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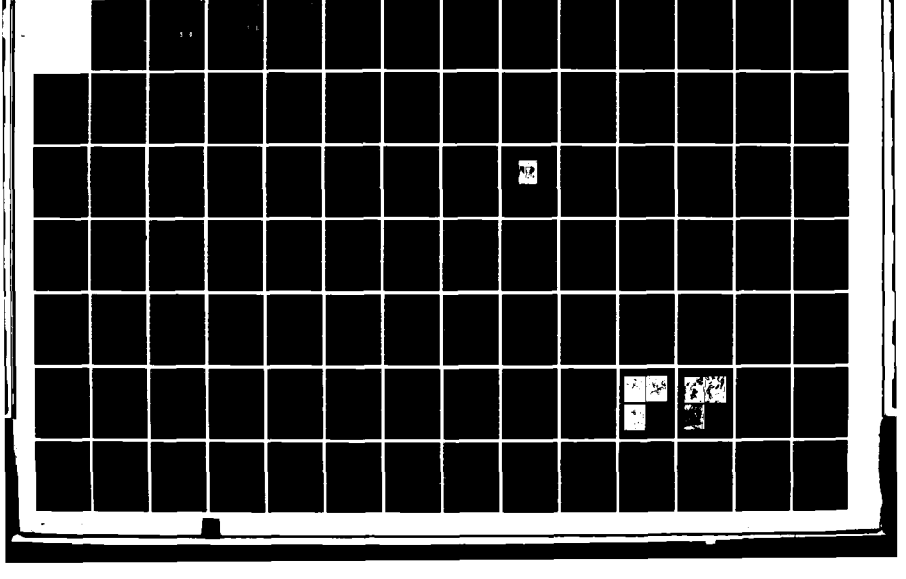

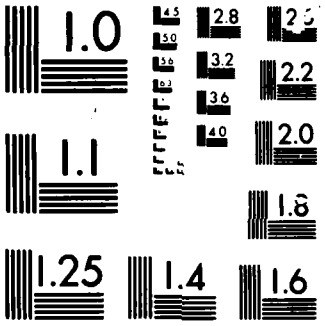

MICROCOPY CHART

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LOCAL ANESTHETIC MICROENCAPSULATION

Final Report

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Local anesthetics have been microencapsulated using a biodegradable polymer, polylactide. Lidocaine base and hydrochlorides of lidocaine, etidocaine and bupivacaine were coated with various quantities of polymer and the microcapsules sieved into various size ranges. In vitro drug release could be varied from immediate release to 25% in one day. Slower release was achieved with larger microcapsules and higher polymer coatings.

Systemic toxicity of selected microcapsules was studied following intraperitoneal injections in mice. Lethal and convulsive doses (LD50 and CD50) were 4.0 to 6.7 times higher with the encapsulated anesthetic hydrochlorides. Peak blood levels in rabbits were significantly lower following I.M. injection of microencapsulated lidocaine and etidocaine than following solution injections. Local toxicity of these microcapsule injections was lower as measured by CPK analysis.

The duration of anesthesia was measured by the blockage of the sciatic nerve of the rat (300-450 gm), using etidocaine-HCl microcapsules (150-212 um, 47% drug content), 50% (contd)

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in vitro release in 23 hours). Most blocks which lasted 12 hours also lasted 48 hours. The median effective dose for this anesthesia (ED50) is approximately 50 mg (150 mg/kg). The CD50 was 330 mg/kg in this experiment. With etidocaine solutions, 48 hours of anesthesia could not be achieved. One day blockage was achieved, but the dose was above the convulsive dose (ED50 > CD50).

I. SUMMARY

Long-lasting local anesthetics have been prepared by microencapsulating lidocaine-HCl, lidocaine (base), etidocaine-HCl, and bupivacaine-HCl with a biodegradable polymer, polylactide. Poly-L(-)lactide was synthesized which has a viscosity (R.S.V.) of 1.2 dl/g, a molecular weight (MWw) of 20,100, and a polydispersity of (MWw/MWn) of 2.14. Drug release precedes polymer degradation in all of these studies.

This polymer was used in air suspension coating equipment to coat the above drug particles. Samples were taken at various coating levels (10 to 50% polymer) and sieved to various size fractions. These materials were analyzed for drug release as a function of time in an agitated phosphate buffer solution at 37°C. Drug release was also correlated to microcapsule morphology (SEM) and surface drug concentration (EDAX). Selected microcapsules were stored under various conditions and analyzed for changes in drug content and rate of drug release.

The rate of drug release decreases with increasing polymer coating, increasing microcapsule size, decreasing drug solubility in water, and decreasing surface drug. Drug release varied from immediate to 25% release in one day (etidocaine-HCl, 50% coating, 06-2-50, 74-300 um). Microcapsules which were chosen for in vivo studies had the following characteristics:

Drug	Run	Drug %	Size (μm)	Bed %	Available Grams	Hours for 50% Release
Lidocaine-Base	11-1-30	75	150-212	8	10	1.5
Lidocaine-HCl	06-1-50	77	150-212	35	70	2.0
Bupivacaine-HCl	11-3-30	64	150-250	50	23	3.5
Etidocaine-HCl	11-2-30	66	106-300	30	58	5
Etidocaine-HCl	06-2-30	(70)	74-106	28	3	7
Etidocaine-HCl	11-4-30	47	150-212	19	36	23

The relative systemic toxicity of solutions and microcapsule suspensions was studied using intraperitoneal injections in mice. Death (LD50) and convulsive (CD50) doses were determined as follows:

		Soln.	M.C.
Lidocaine-HCL 06-1-50, 150-212	LD50	181	840
	CD50	92	490
Etidocaine-HCl 11-2-30, 106-300	LD50	62	260
	CD50	47	190

Bupivacaine-HCl 11-3-30, 150-250	LD50	68	450
	CD50	42	280

The relative safety (LD50 and CD50 ratios) was therefore increased by 4.0 to 6.7 times by microencapsulation.

The relative local toxicity was studied using a creatine phosphokinase assay. Muscle damage of I.M. injections in rabbits was significant when using 4% lidocaine solutions (4100 IU/ml), but not significantly different from the control (2200 IU/ml) when using microcapsules (06-1-50, 150-212 um).

Blood levels of lidocaine were measured by a GC/MS method at USAIDR, an enzyme immunoassay at BIOTEK and two GC/FID procedures at BIOTEK. The GC procedures would also measure circulating etidocaine. Peak levels of both lidocaine and etidocaine were significantly less when injecting microcapsules (vs. solutions) of these drugs, I.M., in rabbits. Very small quantities of circulating lidocaine were found within 24 hours, following injections of lidocaine (base) microcapsules.

Local anesthesia was measured using an EMG response to a mechanical tactile stimulus. The rabbit was found to be an inappropriate model for this analysis, following intradermal or subcutaneous injection of anesthetics. Using a guinea pig model, classical dose response curves were obtained for intradermal wheals. However insufficient quantities of microcapsules could be injected for long term anesthesia, and tissue necrosis was observed with both solutions and microcapsules.

The blockage of the rat sciatic nerve was therefore used as a measure of anesthetic duration. Injections were made into the space between the thigh muscles and the femor. A lack of grasping control was observed for an average of 3 days when using 400 mg/kg (150 mg) of etidocaine-HCl as microcapsules (11-4-30, 150-212). Most blocks which lasted 12 hours also lasted 48 hours, and the median effective dose was 150 mg/kg (at 12, 24, 36, 48 hours, 60, 47, 50, 38% of the rats were anesthetized, respectively). The median convulsive dose was 330 mg/kg for this experiment. For etidocaine-HCl solutions, 24 hours of anesthesia could not be achieved without significant toxicity (CD50 = 50 mg/kg and ED50 = 70 mg/kg for 24 hour anesthesia). Thus the therapeutic index (CD50/ED50) is 2.2 for the microcapsules and 0.7 for the solution, for one day of anesthesia. Also, those microcapsule blocks which lasted one day usually lasted two days. Two days of anesthesia could not be obtained with etidocaine-HCl solutions.

FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

Citation of commercial organizations and trade names in this report does not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

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II. INTRODUCTION

A. OBJECTIVE

The objective of this contract is to develop a long-lasting local anesthetic for application to avulsive maxillofacial wounds. Local anesthetics in standard dosage forms are usually effective for only a few hours. In cases where long-lasting pain relief is necessary, additional anesthetic must be administered. This could be avoided by use of a sustained-release local anesthetic. An effective period of twenty-four hours or more is the objective of this program.

B. MILITARY RELEVANCY

Combat injuries present a different set of problems from civilian injuries seen in a hospital emergency room. In combat, some paramedical support is available in the field. However, the injured soldier may be required to continue fighting or to evacuate the area under his own power. Thus, giving a general analgesic, such as an opiate may not be advisable in the field, and a long-lasting local anesthetic would be advantageous. For less severe injuries a sustained-release local anesthetic in military medicine would allow personnel to perform vital combat functions after receiving injuries which would otherwise require removal from the battlefield.

Paramedical personnel in battlefield conditions could not be expected to deliver an injection in a precise area to generate a nerve block. In the highly vascularized head and neck area it is also possible that this injection of microcapsules could cause embolism. However, for avulsive wounds in the battlefield, the powder can be applied with a dressing or sprayed (with air) onto the wound before the bandage is applied.

Recently, acute pain treatment has been advocated which deliver sufficient analgesic for pain without the patient requesting additional drug. This approach eliminates the learned pain response which so often leads to drug dependence following PRN dosing (U.Washington, School of Medicine, 1983). Pain treatment for soft tissue damage is normally scheduled for 2-3 days. For bone involvement the pain treatment extends for a week or two. Microencapsulated anesthetics would aid in this time-contingent management of acute pain.

The use of biodegradable polymers in this application is advantageous, since fragments of the wound dressing which are left in the wound will be absorbed without incident.

C. BACKGROUND

The duration of local anesthesia is dependent on the time for which the anesthetic is in contact with nerve tissue. Strategies which slowly release anesthetic at the nerve site will prolong the period of anesthesia and reduce the overall quantity of drug required.

A time-release, microencapsulated local anesthetic was developed at Abcor under USAMRDC Contract No. DAMD17-79-C-9019. This anesthetic formulation consists of lidocaine encapsulated with biodegradable polymer coating. Sustained anesthetic action is achievable because the drug diffuses from microcapsules over an extended period of time.

1. Encapsulated Drug Theory

Fick's law of diffusion predicts that if a drug is enclosed in an inert membrane and if the drug concentration within the enclosure is constant and the concentration in the environment outside the membrane is held constant, a steady state will be established in which the diffusion rate of drug through the membrane will be constant with time (Baker and Lonsdale, 1974).

A constant concentration inside the microcapsule may be obtained with a core which is a saturated solution of a drug (and for which undissolved drug is present) or with a core of a pure material.

For spherical microcapsules, the steady state drug release rate is given by:

$$\frac{dm}{dt} = \frac{4\pi \, RoRi \quad DK \, (C\hat{c}-Co)}{Ro-Ri}$$

where:

$\frac{dm}{dt}$ = mass of drug released per unit time

Ri = radius of core

Ro = radius of microcapsule

D = diffusion constant of the drug in the wall material

K = partition coefficient of the drug between the wall material and the surrounding medium (assumed to be the same inside and outside the microcapsule)

Cc = concentration of drug in the core

Co = concentration of drug outside the microcapsule

The steady state release rate may be selected by choice of the parameters on the right hand side of the above equation. If the drug and the wall material are specified, the diffusion constant and the partition coefficient are determined. Co will be determined by the environment of the microcapsule. Thus in designing a drug delivery system with a predetermined drug (core) and wall material, we actually only have at our disposal the geometric parameters, Ro and Ri.

The core of the polylactide-coated microcapsule will consist of a saturated aqueous solution of the drug (water enters the core region by diffusion through the polylactide wall). If it is assumed that Cc is much larger than Co, the rate of release will be proportional to the solubility of the drug. This can sometimes be modified by using different salts of the drug.

The theoretically constant release of drug from a microcapsule system is not achievable in a homogeneous polymer-drug matrix. In these matrices release is slower at longer times because of the decreasing concentration of drug at the outside of the particle.

2. Pharmacology of Local Anesthetics

Since the introduction of cocaine as a local anesthetic by Freud and Koller in 1884 (Ritchie and Grune 1980), local anesthetics have played an important role in clinical medicine.

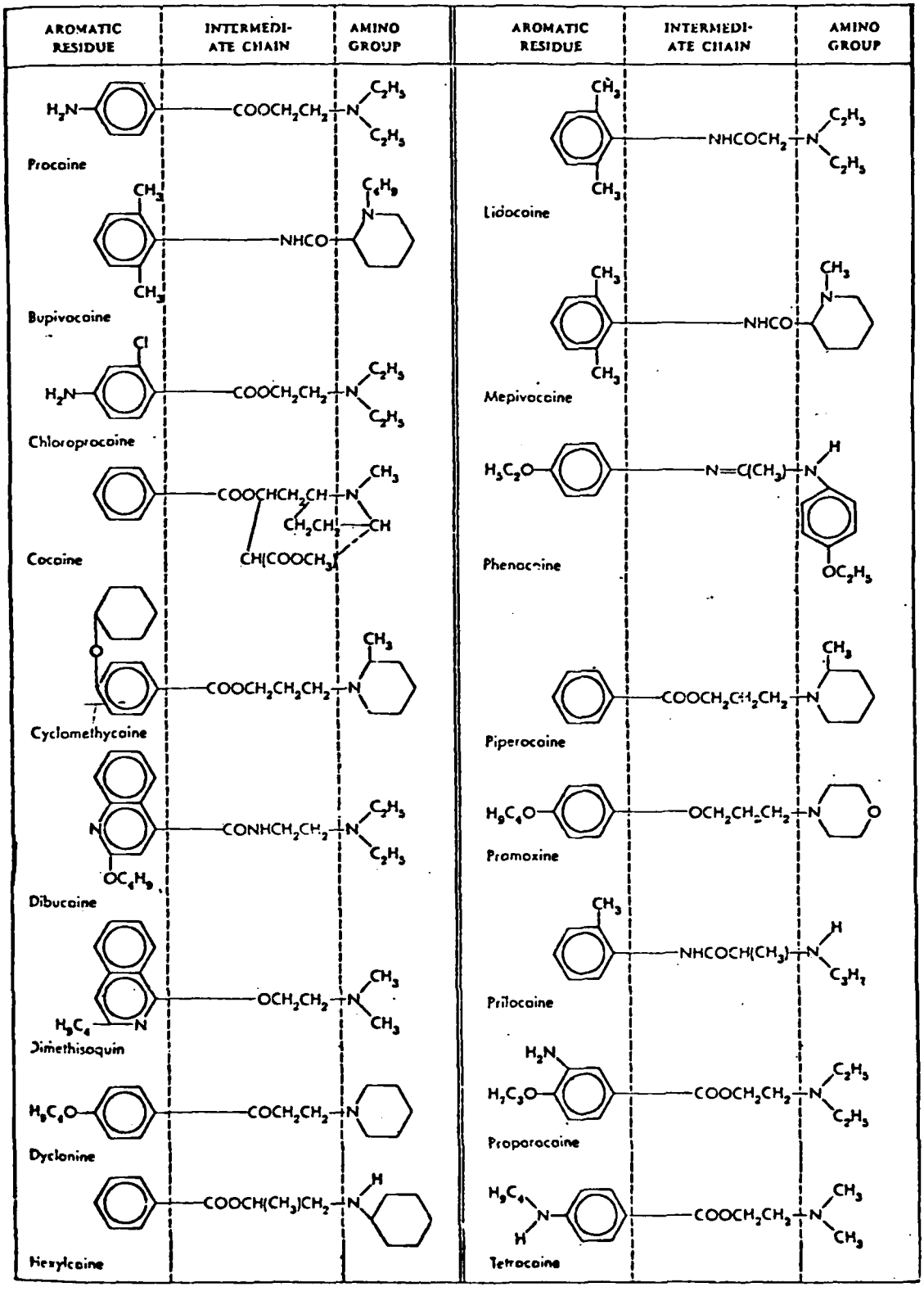
Local anesthetics offer the advantage of allowing surgical anesthesia to be attained while the patient remains fully conscious. Local anesthetics are administered in several ways including topically, by infiltration, and as a nerve block.

Chemical structures of some common local anesthetics are shown in Table 1. The presence of an amine group linked via an amide, ester, or ether intermediate chain to an aromatic ring is characteristic of these anesthetics. The structures of the base forms of the drugs are shown. For injection, the hydrochloride salt is used. Partial conversion to the base form occurs *in vivo* due to the neutral environment and the weak basic properties of the amine group.

TABLE 1

CHEMICAL STRUCTURES OF LOCAL ANESTHETICS

(Source: Ritchie and Cohen, 1975)



a. Action

Local anesthetics block the conduction of nerve action potentials in those nerve fibers and nerve endings exposed to a sufficiently high concentration of the agent. The local anesthetics appear to interfere with the transmembrane movement of sodium and potassium ions which is necessary for the conduction of nerve action potentials (deJong, 1970).

The minimum concentration of anesthetics necessary for complete cessation of nerve action varies according to the size and number of nerve fibers and whether or not they are myelinated (deJong, 1970; Ritchie and Cohen, 1975). Infiltration anesthesia is dependent upon the agents' action on the local nerve endings, and the effect will, be greatest and be of longest duration at the injection site.

The time lapse after injection before the agent takes effect is related to its ease of diffusion through tissue. It has been determined (deJong, 1970) that this diffusion rate is dependent upon the amount of anesthetic base present in the tissues. Since the anesthetics are normally injected as the hydrochloride, the amount of base present is dependent upon the pH and drug pKa (deJong, 1970). Based on these observations, making the preparations more alkaline might increase clinical efficiency, but it was found that such preparations were relatively unstable and that the pH of the local anesthetic was rapidly adjusted to that of the extracellular fluid, regardless of the pH of the injection solution (Ritchie and Cohen, 1975). Since the pKa of most local anesthetics is between 8.0 and 9.0 (lidocaine = 7.9), the body pH of 7.2 will make anesthetics relatively efficient.

b. Localization

In order to decrease the dose required for anesthesia and slow biotransformation of the agent, it is important that the anesthetic be confined to a small region, including the target area, for as long as possible. Since most local anesthetic agents are vasodilators, the result of their injection is increased blood flow to the area, and, therefore, increased absorption into the blood stream. Since amide-linked anesthetics are primarily broken down in the liver, and ester-linked agents are metabolized both in the liver and plasma (Ritchie and Cohen, 1975), increased tissue contact leads directly to increased biotransformation. This also carries the agent away from the target area, decreasing the time at which concentration will fall below the minimum necessary for complete anesthesia.

Anesthetic agents are frequently administered in conjunction with low concentrations of epinephrine or other vasoconstrictors. This not only keeps the agent localized by decreasing blood flow, but decreases the systemic levels of the material to levels that may be quickly metabolized, thus reducing systemic effects. The dosage of vasoconstrictors must, of course, be maintained at as low a level as possible to reduce its systemic effects, and to allow sufficient blood flow to the wound area.

c. Duration

The duration of effect of local anesthetics is dependent upon the period of time which the level of agent remains at, or above, a minimum effective concentration. This period of time can be lengthened by decreasing absorption of the agent into the blood stream (localization) or by increasing or re-administering the dosage.

Anesthetics have been marketed with longer durations than the lidocaine and procaine, which are commonly used, but these agents such as tetracaine and bupivacaine have also been found to be much more toxic (Bennett, 1974, P. 136; Ritchie and Cohen, 1975, P. 389-390). An appealing idea is to formulate an anesthetic agent which exhibits the lower toxicity of lidocaine, while providing much longer duration than has been seen to date for even the long-lasting agents. Microencapsulation offers this possibility.

3. Choice of Local Anesthetic

When using microcapsules of a local anesthetic however, the amount of drug which can be delivered in a given volume is lowered, and the effective concentration of the drug is further lowered by being released over a long period of time. Thus some of the more potent local anesthetics may be necessary for anesthesia in the delayed release system. Also, fewer side effects may be evident because the slow release of the drug prevents high blood concentrations from occuring immediately after application into the open wound.

Many local anesthetics were considered for this program. Lidocaine-HCl, lidocaine (base), etidocaine-HCl, and bupivacaine-HCl were chosen for microencapsulation. Important chemical and physiological properties of these drugs shown in Table 2.

Lidocaine is the most widely used local anesthetic and has a wide therapeutic range. Bupivacaine is a more potent and longer lasting anesthetic, as is etidocaine. Vassallo (p.c., 1980) finds that both drugs are four times more active than lidocaine on isolated tissue (e.g., frog nerve), but that

TABLE 2

LOCAL ANESTHETIC AGENT COMPARISON

(Covino and Vassallo, 1976)

AGENT	Physico-Chemical Properties			Biological Properties		
	partition coefficient [1]	% protein binding [1]	solubility [2]	equi effective anesthetic conc. [3]	approx. anesthetic duration (min) [3]	site of metabolism
Bupivacaine	27.5	95.6	3.9	0.25	175	Liver
Lidocaine	2.9	64.3	240	1	100	Liver
Etidocaine	141	94	24	0.25	200	Liver

1. n-Heptane/pH 7.4 buffer
2. Plasma protein binding - 2 mg/ml
3. Solubility in phosphate buffer mg/ml (Biotek)
4. Data derived from rat sciatic nerve blocking procedure

clinically about twice as much etidocaine is used for pain control. This may be due to lipid solubility differences, or a difference in customary usage which is independent of drug action.

These three local anesthetics have varying solubilities in water, and different distribution coefficients between water and lipoid solvents. The order of decreasing hydrophilicity is etidocaine less than bupivacaine which is less than lidocaine. The hydrochlorides are more soluble than the base form and are more stable in solution. However, the acid salt must be neutralized in the tissues and the free base liberated before the drug can penetrate the tissues and produce anesthesia (Ritchie and Greene, 1980). Since our drug forms are solid systems, we would not expect stability problems of the free base anesthetics.

Salts of lidocaine have been studied by Koehler and Hefferren (1964). They found the hydrochloride solubility to be 1.0 gm/ml (we obtain about 2 gm/ml.). For the phosphate and sulfate 0.29 and 0.25 gm/ml was obtained by Koehler and Hefferren. As would be expected from such soluble salts as lidocaine-HCl, this material is hygroscopic (1 mole H_2O/mole salt at 50% R.H.). The sulfate and phosphate salts were not hygroscopic (Koehler and Hefferren, 1964). The hydroiodide may also have low solubility (Cherney, 1963).

Only the base and hydrochloride forms of the drugs were used in this program. Other materials are not presently described by the U.S. Pharmacopeia.

4. Possible Side Effects of Sustained Release Anesthetic

Lidocaine has been used for many years in many applications and is remarkably free of side effects. Lidocaine infusion is used to supress ventricular arrhythmias and repeated injections are given to relieve pain. For "continuous epidermal or caudal anesthesia, the maximum dose should not be administered at intervals of less than 90 minutes" (P.D.R., 1978). There is no recommended maximum per day or week with this drug. For bupivacaine, doses may be repeated every three hours, and the P.D.R. recommends that daily doses greater than 400 mg should not be used, since they have not been tested.

We do not anticipate a side effect from the long term delivery of these anesthetics into an open wound. Our goal is to replace the 90 or 180 minute injection routine with a more constant delivery of anesthetic by topical application into avulsive wounds. We do not anticipate the use of these materials for any longer periods of time than are presently utilized in repeated application methods.

Toxicity should be considered as a function of too much material delivered too quickly to the patient. If larger quantities are applied than should be used in a single dose (550 mg lidocaine, 175 mg bupivacaine) the slow release of anesthetic must be assured. This can only be assured by animal and clinical testing.

III. ACCOMPLISHMENTS

A. Material Preparation and Selection

1. Polymer Preparation

Poly-L(-)lactide of a reduced viscosity of about 1.0 dl/g has been prepared and used by us for several government contracts. This was the polymer used for the preparation of lidocaine microcapsules by the Wurster process under Contract No. DAMD 17-79-C-9019. It was also the polymer used for much of the steroid encapsulation work performed under Contract No. N01-HD-3-2738. The same method of preparation was used in the present contract.

The preparation of the polylactide was performed as follows: The lactide dimer was obtained from Boehringer-Ingleheim through Henley and Company (N.Y.C., N.Y.). This dimer was purified by repeated recrystallizations from ethyl acetate shortly before use. Reagent Grade solvents were used for all operations. The lactide dimer was heated in a 120°C oil bath while stirring the melt, in vacuum, for 30 minutes to remove traces of volatile materials. An inert gas was then introduced to release the vacuum. Next, the bath temperature was raised to 180°C. To this mixture was added 0.2 ml of stannous octoate catalyst. Within about 15 minutes after the catalyst addition, the polymer mixture reaches a maximum temperature. After about 5 hours, the reaction is stopped by removing the mixture from the oil bath, and the polymer is allowed to cool to room temperature. The polymer block is dissolved in methylene chloride and the solution is decanted and treated with three volumes of isopropanol, by slow addition to a stirred solution.

Two kilograms of poly-L(-)lactide which has a reduced specific viscosity (R.S.V.) between 1.0 and 1.5 dl/g were required for this contract. A parallel contract (DAMD 17-80-C-0110) also required an equivalent amount of polymer of the same R.S.V. To improve the characterization and reproducibility of both programs, it was decided to combine the requirements of both programs in one blend of a number of batches of polymer.

Approximately 1,200 grams of poly-L(-)lactide (R.S.V.=1.0 to 1.2 dl/g) was available from a previous contract (DAMD 17-79-C-9020). This material was used for preliminary experiments on both contracts to quantify operating parameters.

Because of the narrow range of the specified R.S.V. and the excessive heat generated in large batches, preparation of this quantity of polymer was a lengthy process. A total of 12 batches (see Table 3) of dimer were polymerized for these two contracts. Four of these were in the right range and eight

TABLE 3

POLYMERS PREPARED FOR COMBINED PROGRAM

Polymer No.	Starting Grams Dimer	Approximate R.S.V.
5-6-6	574	1.25✓
5-6-10	649	1.02✓
5-6-15	582	0.57
5-6-18	600	2.91
5-6-19	400	> 3.0
5-6-21	665	1.08✓
5-6-23	617	> 3.0
5-6-25	637	2.23
5-6-27	500	0.72
5-6-29	500	2.08+
5-6-30	500	1.57✓
5-6-31	300	0.92
7-12-2	500	1.40✓
7-12-7	500	1.29✓
TOTAL	7,524	

✓ used in blend (total of 3,388 gm)

+ exchanged with NIH program for last two polymers on list

were not. Five of the eight batches were too high and three were too low. Two of the out-of-range batches were exchanged with a similar project which had two batches which fell within our range but which were out of range for that application.

All six batches of polymer were blended together after dissolving in methylene chloride. A total of 19.3 liters of this polymer solution was precipitated by slow addition of isopropyl alcohol in the ratio of 3 to 1 (57.9 liters IPA). Due to the large volumes this had to be carried out in three batches.

After precipitation, the polymer was removed from the solution by vacuum filtration, the pieces were pressed with rubber dam material in the Buchner funnel to remove most of the solvent. The polymer was then placed under vacuum to remove residual solvent. Due to the low volatility of isopropanol and the large volume of polymer, the drying process took two weeks. When the polymer chunks were sufficiently dry, they were ground in a blender to a uniform size, shaken in a large bag and again placed under vacuum to complete the drying. Three random samples of this final mixture were taken and their viscosities determined in duplicate. The viscosity was found to be 1.19 $\pm$0.03 dl/g and the total polymer obtained was 3,048 grams which was a 90% yield.

The polymer was stored under argon in the freezer. The polymer was placed in plastic bags into one-gallon cans which are tightly sealed (paint cans). The air was removed by vacuum and replaced by argon, just prior to sealing. This procedure has been standardized, but is presumably much more stringent than necessary to prevent polymer degradation.

B. Polymer Characterization

A sample of polymer was sent to Cambridge Analytical Associates for determination of molecular weight distribution by gel permeation chromatography. Samples were dissolved in hot trichlorobenzene. Duplicate injections of the samples were run 12 hours apart to determine whether the high temperature analysis would degrade the polymer. After 12 hours, no evidence of polymer degradation was evident. A series of Micro-Styragel columns (10^3, 10^4, 10^5, 10^6 A) from Waters Associates were used at 145°C. The flow rate was 1.5 ml/min. and the change of refractive index was used for detection. The molecular weight determination was made by reference to polystyrene standards.

The data of molecular weight distribution was calculated by Cambridge Analytical Associates, based on the polystyrene standards curve for the gel permeation chromatography (GPC) columns. A more appropriate calculation is based on the relative size of the polylactide and polystyrene molecules. The standard value for polystyrene is 41 atomic weight units per angstrom. For polylactide, a calculation using standard bond lengths and angles yields 18 atomic weight units per angstrom. The molecular weight data based on this calculation is given in Table 4. This weight-average molecular weight (MWw) is in agreement with the data of Nuwayser, et al (1976), see also Figure 1.

Further analysis of the GPC peak indicates that the actual molecular weight distribution is quite broad. The raw data of refractive index of the collection tubes was analysed as a Gaussian curve for a similar polymer. The mean MWw was 34,400 (polydispersity 2.33). At $\pm$1 S.D. from this mean, the molecular weights are 7,200 and 50,400. This analysis indicates that the broadening of the molecular weight distribution by mixing polymers of different viscosity ($\pm$ 0.5 dl/g) may be insignificant compared with the distribution within a single batch of polymer.

This broad molecular weight distribution was also found to be natural for the process. The theoretical polydispersity of a completed condensation polymerization reaction is 2.0 (Flory, 1953). The polydispersity (MWw/MWn) of our polymer blend is 2.14.

To prove that batches with widely varying viscosities could be blended, a sample of 0.73 dl/g polymer (5-6-27) and 2.04 dl/g polymer (5-6-25) was analyzed by gel permeation chromatography. Also a combination of 33% of the low and 67% of the high molecular weight polymer was analysed. The results are summarized in Table 5 and compared with the initial polymer analysis of Contract No. DAMD 17-80-C-0110. At equal concentrations of the two polymers (0.73 and 2.04 dl/g) the chromato-

TABLE 4

POLYMER MOLECULAR WEIGHT DISTRIBUTION

Sample: Poly-(L-)lactide (R.S.V. = 1.19 dl/g)	
Number Ave. MW (MW_n)	9,440
Weight Ave. MW (MW_n)	20,100
Polydispersity (MW_w/MW_n)	2.14

FIGURE 1

CORRELATION BETWEEN WEIGHT AVERAGE MOLECULAR WEIGHT OF POLYLACTIDES AND REDUCED SPECIFIC VISCOSITY (Nuwayser, et al, 1976)

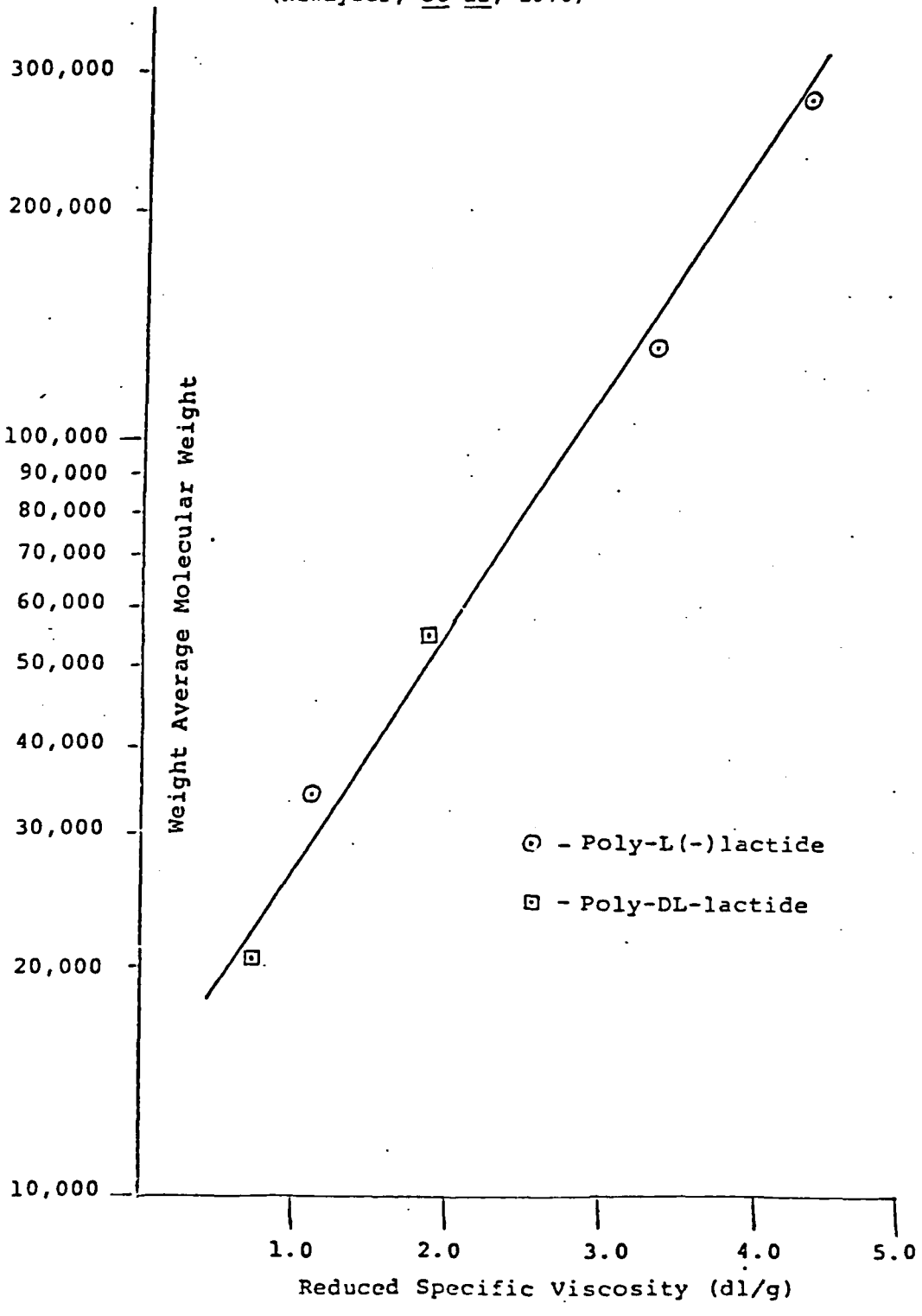


TABLE 5

EFFECT OF VISCOSITY (R.S.V.) ON POLYMER BLENDS

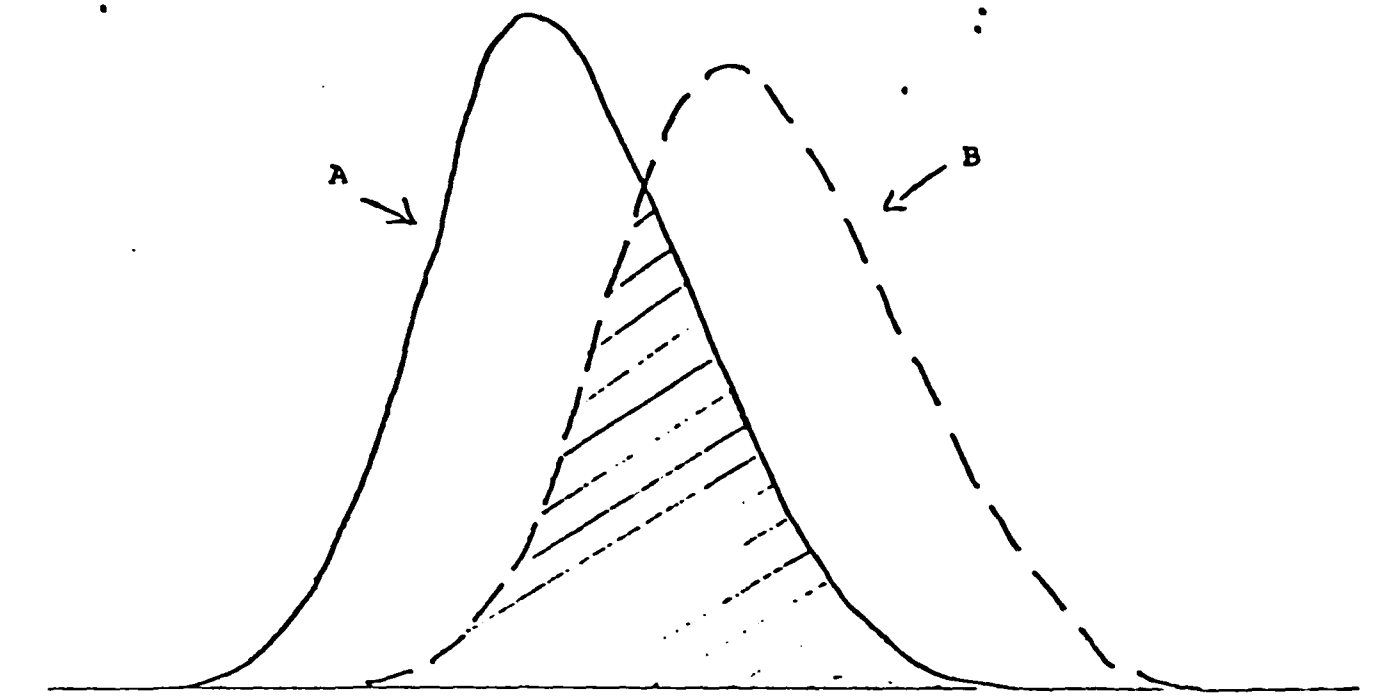
R.S.V.	Polymer	MWw	MWn	MWw/MWn
0.73	5-6-27	29,880	17,590	1.70
2.04	5-6-25	87,840	52,020	1.69
1.20*	Blend of Above	57,420	22,860	2.51
1.19	Previous Contract Blend	45,700	21,500	2.14

* calculated R.S.V.

gram overlap is 48% (Figure 2). The combination chromatogram does not show a bimodal distribution but does show a tailing at high molecular weights.

Thus we can probably blend this wide a viscosity range without affecting the polymer coating and drug release properties of the polymer blend.

For Contract DAMD17-81-C-1195 we indicated that the use of poly-L(-)lactide of R.S.V. = 1.2 dl/g from batches of R.S.V. between 1.0 and 1.5 dl/g was unnecessarily restrictive. Since the polymerization process produces a wide range of molecular weights, a range of 1.2 $\pm$0.6 was proposed. However, no additional material was needed for this contract.



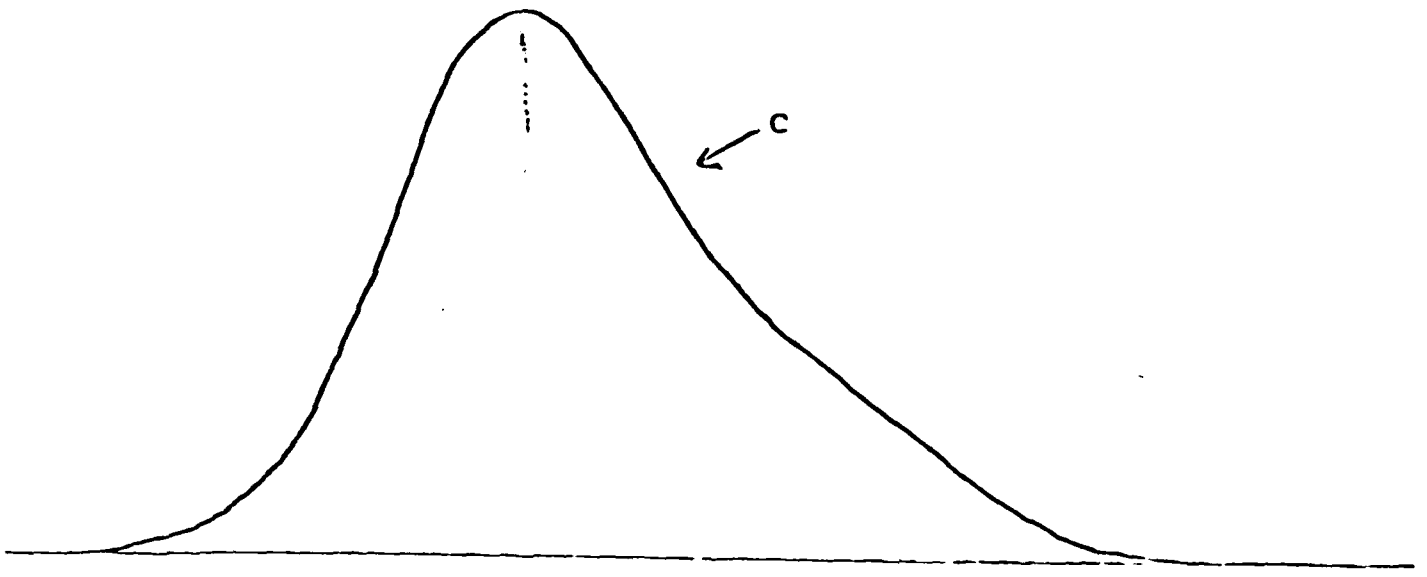


Figure 2 Gel Permeation Chromatograms of
A. 5-6-27, R.S.V. = 0.73 Pd = 1.70
B. 5-6-25, R.S.V. = 2.04 Pd = 1.69
C. 2 of 5-6-27 and 1 of 5-6-25
R.S.V. = 1.2 Pd = 2.51

C. DRUG PROCUREMENT

Because of the nature of the encapsulation process, kilogram quantities of each anesthetic was required.

Lidocaine hydrochloride and base were commercially available from Sterling Organics (Div. of Sterling Drug, N.Y.C., N.Y.). Material oonforming to USP standards was purchased.

Etidocaine hydrochloride was graciously donated for the project by Astra Pharmaceutical Products, Inc. (Worcester, Mass.). This drug was stipulated for use in laboratory research animals or in vitro tests (i.e., not for use in humans). It was labelled as Duranest hydrochloride, Control No. 11P. A smaller quantity of etidocaine base was also donated by Astra Pharmaceuticals.

Bupivacaine was unavailable to us from Breon Laboratories (Div. of Sterling Drug). It was also unavailable from Bofors-Lakeway in this country. Several companies were contacted that will synthesize specific chemicals. Calls were placed to several such companies, giving details of the patent procedures (e.g., U.S. Patent 2,955,111, 1960, assigned to Bofors), and suggesting an alternate route using commercially available starting materials (2-pipecolinic acid and 2,6-dimethylaniline). Other companies were contacted which advertise worldwide connections for pharmaceuticals and other chemicals.

Chem Biochem Research, Inc., Salt Lake City, Utah, was chosen to synthesize the drug and a purchase order was placed. The synthesis proceeded without incident and Dr. Sweat (Chem Biochem Research) stated that the IR and NMR analyses were consistent with bupivacaine and that TLC indicates an absence of impurities found in a commercial sample (Marcaine^{R}).

D. MICROENCAPSULATION

The microencapsulation process is basically the same as described by Wurster (1959). The equipment was purchased from Dairy Equipment Corporation, Madison, Wisconsin and is shown in Figure 3. It has been modified to allow coating of smaller particles.

The first microencapsulation of this program was made with lidocaine-HCl, while we were in Wilmington, Mass. After the equipment was moved to Woburn and reassembled, etidocaine was microencapsulated. Both runs followed the general method used for L-8(50%) of Contract No. DAMD17-79-C-9019. In this previous run, material from two separate 10% polymer runs were blended as the starting material for L-8. In the present experiments, the runs were continued after removal of the oversized material.

1. Lidocaine-HCl Microencapsulation (Run 2106-1)

For lidocaine, the material was Sterling, Lot N111RF. Methylene chloride was Baker Reagent, Lot 025824. the coating polymer solution concentration was 3% (w/v). The solution was added at 20 ml/min. up to a loading of 10% polymer on drug. The spraying rate was then dropped to 4.5 ml/minute. A 2850/50 Spraying Systems nozzle was used throughout, as in the previous contract work. The atomizing pressure was maintained at 25 psi, as in the previous work.

Table 6 shows the processing losses. Oversize material was removed at each coating level, as shown in this table. Equipment holdup, losses, and sample removal lowers the yield from that expected from a continuous operation. However, the overall yield is 27.5%, of 50% coated drug in microcapsules of less than 250 microns.

Table 7 shows the size range of microcapsules at each of the coating levels. In the initial coating stage, considerable agglomeration occurs. This is the reason for sieving the material at 150 microns after about 10% polymer is added. Thereafter the material is more uniform and spherical. This leads to less agglomeration, although the particle size continues to rise at a faster rate than expected for overcoating of a constant number of particles.

2. Etidocaine Microencapsulation (Run 2106-2)

During the reassembly of the Wurster unit, several improvements were made in the system. However, for comparison of etidocaine to lidocaine, it was important to change as few processing variables as possible. Etidocaine-HCl was used as

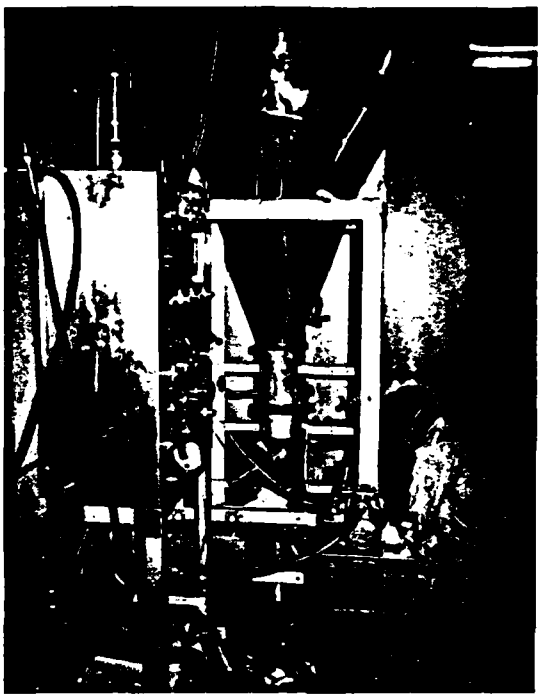


Figure 3. Air Suspension Coating Equipment for Anesthetic Microencapsulation

TABLE 6

PROCESSING SUMMARY OF LIDOCAINE MICROENCAPSULATION

(Run 2106-1)

Process Polymer %	Starting Sample g	Polymer Added g	Final Weight MC g	Final Weight (Drug) (g)	Losses Equipment Holdup g	Losses Oversize Removal g at (μm)	Samples Removed g
0-7	400	30	259	(238)	92	69 (150)	10
7-20	259	33	275	(220)	6	1 (250)	10
20-30	275	40	273	(191)	13	19 (250)	10
30-40	273	43	259	(155)	10	37 (250)	10
40-50	259	51	220	(110)	0	63 (250)	27 (220)
TOTALS		197			121	189	287

Material Balance: (in) 400 g (drug) + 197 g (polymer) = 597 g
(out) 287 g (samples) + 310 g (losses) = 597 g

Yield at 50% Coating (< 250 μm): 110:400 = 27.5%

TABLE 7

LIDOCAINE MICROCAPSULE SIZE DISTRIBUTION

(Values are % of weight in each sieve fraction)

Particle Size Range (μm)	% Drug in Microcapsule				
	93	80	70	60	50
> 212	24.6	0.9	13.9	25.1	24.2
150-212	6.3	13.8	20.9	26.3	34.6
125-150	8.2	17.6	13.3	17.4	11.4
106-125	17.4	35.5	10.5	11.6	11.9
38-106	40.1	30.4	36.1	14.6	6.8
< 38	3.4	1.9	5.6	5.0	11.7

received from Astra Pharmaceuticals Products (Lot 11P). In this experiment we had better control and accounting procedures for losses.

At the 50% coating level a large sample was removed (110 gm) and the remaining material was coated with an additional 10% of polymer, as rapidly as possible, The coating procedure was successful, indicating that the equipment can maintain a fluidized bed with only 165 grams in the coating chamber. Furthermore, the rate of solution addition was raised slowly to 10 ml/minute without having the bed collapse. The critical processing indicator is negative pressure at the nozzle. At the temperature of these experiments (38°C at the nozzle), a flow of 10 ml/minute is practical.

The summary of the processing losses is shown in Table 8. The sieve analysis of these materials is shown in Table 9.

3. Bupivacaine Microencapsulation (Run 2106-3)

Bupivacaine was microencapsulated using the same procedure as was used for etidocaine, except that the coating was stopped at 50% of added polymer. The processing summary is shown in Table 10. Table 11 shows the size distribution of the microcapsules obtained.

4. Microencapsulation Goals of new Contract

Initial encapsulations of lidocaine, etidocaine, and bupivacaine hydrochlorides were performed on Contract DAMD17-80-C-0110. Based on these experiments, the goals of the next encapsulations were to:

a. prepare bupivacaine and etidocaine hydrochloride microcapsules to 30% polymer, coating in a more time-efficient manner,

b. using a less soluble form of lidocaine, prepare microcapsules which will release the drug more slowly.

5. Lidocaine (Base) Microencapsulation (Run 11-1)

A Wurster run was attempted with lidocaine (base). The material, as received from Sterling Organics, Inc., was in chunks which could be broken with finger pressure. There was also some material in this sample which was not soluble in methylene chloride. Since the material could not be fluidized, as received, we purified it by removing the material which was insoluble in methylene chloride, and prepared crystals by evaporation of the methylene chloride. The final product appeared to flow better, and it was placed in the Wurster coating cham-

TABLE 8

PROCESSING SUMMARY OF ETIDOCAINE MICROENCAPSULATION

(Run 2106-2)

Process Polymer %	Starting Sample g	Polymer Added g	Final Weight MC g	Final Weight (as drug) (g)	Wurster Holdup g	Oversize Removed g (μm)	Sieve + Bag g	Samples Removed g
0-11	400	49	385	(343)	64	23 (150)	13	10
11-20	338	31	332	(266)	37	1 (250)	4	10
20-30	316	40	311	(218)	45	2 (250)	2	10
30-40	297	42	314	(188)	25	48 (250)	3	10
40-50	251	50	275	(138)	26	20*(250)	2	110
50-60	165	40	201	(80)	4	35*(250)	2	(201)
TOTALS		252			201	74	26	351

Material Balance: (in) 400g drug + 252 g polymer = 652 g
(out) 351 g (samples) + 201 (Wurster) + 74 (oversize) + 26 (bag) = 652 g

Yield of material at 50% polymer: 138 g/400 g = 34.5%
30% polymer: 218 g/400 g = 54.5%

* Based on sieve analysis of sample, material not actually removed

TABLE 9

ETIDOCAINE MICROCAPSULE SIZE DISTRIBUTION

(Values are % of weight in each sieve fraction)

Particle Size Range (μm)	% Drug in Microcapsule 80	70	60	50	40
>300	2.2	1.9	6.5	4.0	4.3
212-300	3.3	5.5	15.4	15.8	26.7
150-212	15.2	20.3	24.2	34.2	41.2
106-150	26.5	21.9	28.2	23.9	18.7
74-106	17.2	28.5	9.3	12.1	7.1
38-74	33.5	21.2	15.8	9.8	1.8
< 38	2.1	0.7	0.5	0.1	0.1

* 40% sample is removed from base of coating chamber. Other samples are total (i.e., after brush-down of equipment, expansion chamber, filters, rotating nozzles (tree), sides of coating chamber, etc.)

Table 10

PROCESSING SUMMARY OF BUPIVACAINE MICROENCAPSULATION

(Run 2106-3)

	Starting Weights				Losses			
Process Polymer %	Starting Sample g	Polymer Added g	Final Weight MC g	(as drug) (g)	Wurster Holdup g	Oversize Removed g (250μm)	Sieve + Bag g	Samples Removed g
0-10	400	45	401	(361)	44	111	15	5
10-20	270	34	289	(231)	15	10	15	14
20-30	250	36	280	(196)	6	31	5	46
30-40	198	32	223	(134)	7	31	22	40
40-50	130	26	178	(89)	-22	36*	10	(168)
TOTALS		173			50	183	67	273
	A	B	C		D	E	F	G

Material Balance: (in) 400 g (drug) + 173 g (polymer) = 573 g
(out) 273 (samples) + 50 (Wurster) + 183 (oversize) + 67 (bags, etc.) = 573 g

* Based on sieve analysis of sample, material not actually removed

$D_n = A_n + B_n - C_n$ $\qquad A_{n+1} = C_n - (E_n + F_n + G_n)$

TABLE 11

BUPIVACAINE MICROCAPSULE SIZE DISTRIBUTION

(Values are % of weight in each sieve fraction)

Microcapsule Size Range (μm)	% Drug in Microcapsules			
	80%	70%	60%	50%
> 250	1.3	1.7	2.5	3.8
212-250	15.4	20.8	30.5	35.4
150-212	28.3	30.0	33.1	25.3
106-150	20.2	14.0	10.2	11.3
75-106	12.9	14.1	9.9	13.7
38-75	16.4	12.1	10.1	7.9
< 38	5.5	7.3	3.6	2.6

ber. However, it was not possible to produce a fluidized bed for coating purposes. Other solvents and drying procedures were therefore investigated and hexane was found to be the best solvent for recrystallization.

Lidocaine salts can be prepared by precipitation of the less soluble salt in water or by addition of the appropriate acid to the base form of the drug. Best results were obtained by dissolving the lidocaine (base) in ethanol and adding concentrated sulfuric or phosphoric acid solutions (aqueous). The precipitates were then washed with ethanol. Lidocaine sulfate has a melting point of approximately 215°C and is slightly soluble in water. Lidocaine phosphate has a melting point of approximately 180°C, but it is very soluble in water. Thus the sulfate would be the salt of choice for encapsulation. Since lidocaine (base) is extremely soluble in methylene chloride we were also concerned that the coating process might agglomerate the bed. Thus we were prepared to use the sulfate.

Previous Wurster coatings were started at 20 ml/minute to agglomerate the drug particles. At 10% polymer coating, the coating rate had been reduced to 3 ml/minute. However, in this run, severe agglomeration occurred at 10 ml/minute, and the bed collapsed. the oversized material was ground through a Braun mill and placed back in the Wurster unit. Coating continued at 5 ml/minute and was stopped at 30% polymer. This required only two days of coating time. For comparison, the coating of lidocaine-HCl to 50% polymer had taken five days.

The summary of the results of the coating process is shown in Table 12. The microcapsule size distribution is shown in Table 13. The microcapsules are larger than had been expected, and there are considerable fines (brushed sample) which have a high drug content.

6. Etidocaine Microencapsulation (Run 11-2)

Etidocaine hydrochloride was again microencapsulated. The same materials were used as in the microencapsulation of the previous contract. However, the method of microencapsulation was similar to that described for lidocaine (base) above.

Etidocaine-HCl fluidizes more readily than does lidocaine (base). The agglomerating phase in which the 3% polymer solution is introduced at 20 ml/minute (0-11% coating) caused only a slight agglomeration of particles. Particles larger than 250 microns (51 grams) were removed from the bed (see processing summary, Table 14). The coating was then continued at 10 ml/minute; but even this coating rate did not significantly increase the mean particle size. The entire coating time to

TABLE 12

PROCESSING SUMMARY OF LIDOCAINE (BASE) MICROENCAPSULATION

(Run 11-1)

	Starting Weights		Final Weight		Losses			
Process Polymer %	Starting Sample g	Polymer Added g	MC (as drug) g	(g)	Wurster Holdup g	Oversize Removed g (500μm)	Sieve + Bag g	Samples Removed g
0-13	300	33	186	(165)	148	ground	-	10
13-20	176	15	198	(165)	-7	55	1	30
20-30	112	16	122 84*	(158)**	6 -84*	-	-	122 84*
TOTALS		64			63 -	55	1+	246
	A	B	C		D	E	F	G

Material Balance In: 300g drug + 64 g polymer = 364 g
Out: 246g samples + 163 Wurster + 55 oversize = 364 g

** Yield at 30% is 53% (not considering 40 g of samples) based on drug

** Yield at 30% is 68% based on samples/input material

$D_n = A_n + B_n - C_n$ $\quad A_{n+1} = C_n - (E_n + F_n + G_n)$

* Brushed down of Wurster unit yields 84 grams
** Includes brushed-down material

TABLE 13

LIDOCAINE (BASE) MICROCAPSULE SIZE DISTRIBUTION

(Values are % of weight in each sieve fraction)

Microcapsules Size Range (μm)	% Drug in Microcapsules 90%	80%	70%*
> 600	10	0	3
425-600	18	20	22
300-425	17	24	28
212-300	16	20	23
150-212	11	9.2	7.9
106-150	9.1	7.3	4.7
75-106	10.4	8.7	5.4
38-75	8.5	8.8	5.1
< 38	0.9	1.1	0.5

* Does not include brush-down material

TABLE 14

PROCESSING SUMMARY OF ETIDOCAINE·HCl MICROENCAPSULATION
(Run 11-2)

Process Polymer %	Starting Weights: Starting Sample g	Starting Weights: Polymer Added g	Final Weight: MC g	Final Weight: (as drug) (g)	Losses: Wurster Holdup g	Losses: Oversize Removed g (500μm)	Losses: Sieve + Bag g	Losses: Samples Removed g
0-11	300	36.5	203	(181)	133.5	44 51 (>250)	4	11
11-20	137 (<250)	15.5	176	(141)	-23.5	6	0	11
20-30	159	25.5	195 33*	(136) (23)	-10.5	-	-	195 33*
TOTALS		77.5			66.5	57	4	250**
	A	B	C		D	E	F	G

Material Balance In: 300 g drug + 77.5 g polymer = 377.5 g
Out: 250g samples + 57 g oversize + 66.5 g Wurster holdup + 4 g other losses = 377.5 g

**Yield at 30% is 53% based on drug (not including samples purposefully removed)
**Yield at 30% is 66% based on samples/input materials

$$D_n = A_n + B_n - C_n \qquad A_{n+1} = C_n - (E_n + F_n + G_n)$$

* Brush down of Wurster unit yields 33 grams
** Includes brushed-down material

reach the 30% coated sample was 3.4 hours. Almost 20 hours of coating was required for the previous etidocaine microencapsulations, with 10 hours being required to reach 30% polymer.

The size distribution of the microcapsules is shown in Table 15. This distribution is more similar to the previous etidocaine run that the lidocaine (base) run. However, some large particles were available for testing.

7. Bupivacaine Microencapsulation (Run 11-3)

Bupivacaine hydrochloride from Chem Biochem Research was encapsulated in the same manner as described above. The material was intermediate in flowability between the sticky lidocaine (base) and the flowable etidocaine-HCl. The charge of 300 grams of drug was fluidized and coated with 3% polymer solution at 10 ml/minute. Some agglomeration occurred and 73 grams of particles greater than 500 microns were removed after 9% coating and ground in the Braun mill. This material was returned to the system for further coating. The coating was continued at 10 ml/minute to achieve a 20% and 30% coating, based on the bed weight at the start of each increment of coating. The total coating time was 2.8 hours.

The processing summary is shown in Table 16, and the size distribution in Table 17. There is a wide distribution of size ranges available for testing.

8. Microencapsulation of Etidocaine-HCl (Run 11-4)

In previous microencapsulations, etidocaine-HCl particles fluidized so well that agglomeration did not occur, even at high fluid flow rates. Therefore, this time a slurry of drug particles was suspended in a solution of polymer in methylene chloride, and the solvent was evaporated. The resulting film was broken up and the particles forced through a 500 micron and then a 250 micron sieve. A 15% polymer loading was anticipated. However, the final product had an assay value of 22.4%, indicating a loss of fine drug particles. This material made an excellent core particle for fluidized bed spray coating. A large fraction of the product was in the 100-400 micron size range. Table 18 summarizes the process yields, and Table 19 gives the sieving analyses of these materials.

TABLE 15

ETIDOCAINE·HCl MICROCAPSULE SIZE DISTRIBUTION

(Values are % of weight in each sieve fraction)

Microcapsules Size Range (μm)	% Drug in Microcapsules 90%	80%	70%*
>600	25***	-	1.1
425-600	0.7	-	2.1
300-425	0.4	7.6**	6.4
212-300	3.0	5.2	7.8
150-212	14.2	12.5	10.9
106-150	19.4	20.2	18.8
75-106	31.7	31.6	24.5
38-75	3.2	21.1	27.3
<38	2.3	1.8	1.1

* Does not include brush-down material

** >300 μm, small sample

*** Removed from bed

TABLE 16

PROCESSING SUMMARY OF BUPIVACAINE·HCl MICROENCAPSULATION (Run 11-3)

	Starting Weights		Final Weight		Losses			
Process Polymer %	Starting Sample g	Polymer Added g	MC (as drug) g	(g)	Wurster Holdup g	Oversize Removed g (500 µm)	Sieve + Bag g	Samples Removed g
0-9	300	30	136	(123)	194	0[3]	21	10[4]
9-20	105	13.8	-	-	-	-	-	41
20-30	78	6.6	200[2]	(140)	-115	-	-	68 132[1]
TOTALS		50.4			79	0	21	251
	A	B	C		D	E	F	G

Material Balance In: 300 g drug + 50.4 g polymer = 350.4 g
Out: 251 g samples + 0 g oversize + 79 g Wurster holdup + 21 g other losses = 351 g

[2]Yield at 30% is 47% based on drug (not including samples purposefully removed)
[2]Yield at 30% is 72% based on samples/input materials

$$D_n = A_n + B_n - C_n \qquad A_{n+1} = C_n - (E_n + F_n + G_n)$$

[1] Brushed down of Wurster unit yields 132 grams
[2] Includes brushed-down material
[3] 73 g of > 500 µm ground and returned to bed
[4] Sample of ≤ 250 µm material

TABLE 17

BUPIVACAINE·HCl MICROCAPSULE SIZE DISTRIBUTION

(Values are % of weight in each sieve fraction)

Microcapsule Size Range (μm)	% Drug in Microcapsules 90%***	80%	70%*
>600	-	0.5	0.5
425-600	-	9.4	11.3
300-425	3.0**	18.4	18.1
212-300	12.3	19.5	19.4
150-212	20.4	17.7	15.6
106-150	19.5	15.1	15.8
75-106	19.5	8.8	14.3
38-75	17.1	6.4	4.3
<38	6.8	2.1	2.6

* Does not include brush-down material

** > 300 μm, small sample

*** Does not include 73 grams of > 500 μm which was returned to the bed

TABLE 18

PROCESSING SUMMARY OF ETIDOCAINE·HCl MICROENCAPSULATION

(Run 11-4)

Coating Polymer (%)	Starting Weights: Starting Sample (g)	Starting Weights: Polymer Added (g)	Final Weight of Microcapsules (g)	Losses: Wurster Holdup (g)	Losses: Oversize Removed (g)	Losses: Sieve + Bag (g)	Losses: Samples Removed (g)
0-10	280[3]	31	190	121	0	-	30
10-20	160	18	182	-4	0	-	30
20-30	152	17	241[2]	-72	0	-	191 circulating 50[1] brushed
TOTALS		66		45	0	0	301[2]
	A	B	C	D	E	F	G

Material Balance In = 280 g core + 66 g polymer = 346 g

Out = 301 g samples + 45 g losses = 346 g

Yield at 30% coating is 87% based on samples/input materials[2]

$D_n = A_n + B_n - C_n$ $\qquad$ $A_{n+1} = C_n - (E_n + F_n + G_n)$

[1] Brushing of chamber yields 50 grams

[2] Includes brush-down material

[3] Core particles were 22% polymer, < 250 μm

TABLE 19

ETIDOCAINE·HCl MICROCAPSULE SIZE DISTRIBUTION
(Run 11-4)
(Values are % of weight in each sieve fraction)

Microcapsule Size Range (μm)	Core Material	% Coating on Core		
		10%	20%	30%*
> 600	--	0	0	1
425-600	--	1	2	3
300-425	--	9	16	24
212-300	17	25	28	25
150-212	28	20	21	19
106-150	32	17	15	14
75-106	9	3	10	6
38-75	0	19	5	7
< 38	14	4	2	2

* Does not include brush-down material

E. *IN VITRO* DRUG RELEASE STUDIES

Drug release rates are determined by suspending a known mass of microcapsules in a known volume of an aqueous solution and periodically measuring the concentration of the drug in solution by spectrophotometry. From the solution volume and concentration, the quantity of drug released is computed. This quantity divided by the length of time since the last measurement is the average drug release rate over the time interval.

The suspending solution for these studies is water buffered with phosphate (pH 7.4 Sorenson's buffer, 0.05 molar). These solutions are thermostatted at 37°C in a metabolic shaker bath. The vessel used for the release studies is a specially designed L-shaped test tube. The shape of this container promotes good mixing of the release solution when used in a metabolic shaker and thereby reduces local drug concentration gradients in the solution, which might affect the release rate. The microcapsules are placed in a tea-bag type structure, constructed from fine polyester mesh for convenience in separating the microcapsules from the suspending solution. Lidocaine, etidocaine, and bupivacaine have absorption bands in the ultraviolet, which are used to monitor concentrations of the drug in solution.

As a screening procedure, one sieve fraction (106-125 microns) of each percent coating level is used for drug release studies, as well as several of the sieve fractions of samples of one coating level. Samples showing potentially useful release rates are re-examined to give triplicate data.

Late in the program BIOTEK installed a small computer facility and the *in vitro* diffusion data of this program has been entered on computer disc for ease of entry, storage, sorting, and graphing. We are using a LOTUS 1-2-3 worksheet program which is available for our IBM Personal Computer. This package also includes a GRAPH-program disc.

An appendix to this report includes a printout of the *in vitro* drug release data. Drugs and storage conditions have been sorted first by drug, then by nominal drug loading, followed by smallest particle size, and then by storage condition. Data without dates precedes the present contracts.

1. Lidocaine-HCl Release Studies (Run 06-1)

Lidocaine is very soluble in pH 7.4 buffer and samples can be removed, read at 262 nm and returned to the diffusion cell. Because of the microencapsulation process, the percent drug may not be the same for all sizes of particles. Therefore, samples of each size range are assayed for percent drug, and the percent of drug released is based on this assay value.

The results of the drug release studies for the first series of experiments are shown in Table 20. Since relatively slow release was obtained only with the largest microcapsules which were coated to 50%, these microcapsule tests were repeated. Table 20 and Figure 4 show the results of these diffusion experiments. A sample of the 150-212 micron microcapsules was sent to Colonel Posey on February 27, 1981, for use in in-house animal studies.

Next, larger microcapsules were sieved and tested for rate of drug release. The larger, 250 to 300 micron, microcapsules have significantly slower release rates. Whether this release would be even slower in vivo and would give sufficient anesthesia for several additional hours could only be determined by animal studies.

2. Etidocaine-HCl Release Studies (Run 06-2)

Etidocaine is less soluble in water and buffer than is lidocaine. The lipophilic nature of the drug is used to an advantage in maintaining the drug at the site of injection for a longer period of time. The partition coefficients for the various drugs between n-heptane and buffer (pH 7.2), are:

Drug	K
lidocaine	2.9
etidocaine	141
bupivacaine	27.5

(Covino and Vassallo, 1976). Acidifying the buffer solution was suggested (Vassallo, p.c.) prior to making ultraviolet absorption measurements. This procedure was followed but it does not negate the requirement to change the buffer solution as the drug reaches the saturation value.

Figure 4 shows the effect of polymer loading on the drug release characteristics for 212 to 310 micron microcapsules. These large microcapsules were chosen because of the fast release of small lidocaine microcapsules. Figures 6 and 7 show the effect of size on the 50% and 40% drug microcapsules. With this information a drug release study was performed using small microcapsules containing as much as 70% drug. This release approximates the in vivo targeted release (Figure 8).

3. Bupivacaine-HCl Release Studies (Run 06-3)

Bupivacaine is only slightly soluble in water. Therefore the same method of acidification and discarding of samples was used as for etidocaine. Table 21 shows the release characteristics of selected microcapsules. From this data we would

TABLE 20

LIDOCAINE RELEASE FROM MICROCAPSULES

(Run 06-1)

a) Effect of Sizes and Coating Levels

Size Range (μm)	Coating Values	Assay Value	% Released at Hours 1	2	4	6	24
106-125	93	95.3	92.8				
106-125	80	82.0	99.7				
106-125	70	79.6	81.9				
106-125	60	73.9	93.0	94.7			
106-125	50	49.6	88.4	90.8	95.1	97.0	
125-150	50	46.0	68.8	78.8	84.2	87.4	89.7
150-212	50	46.7	43.7	56.2	71.6	79.2	83.7

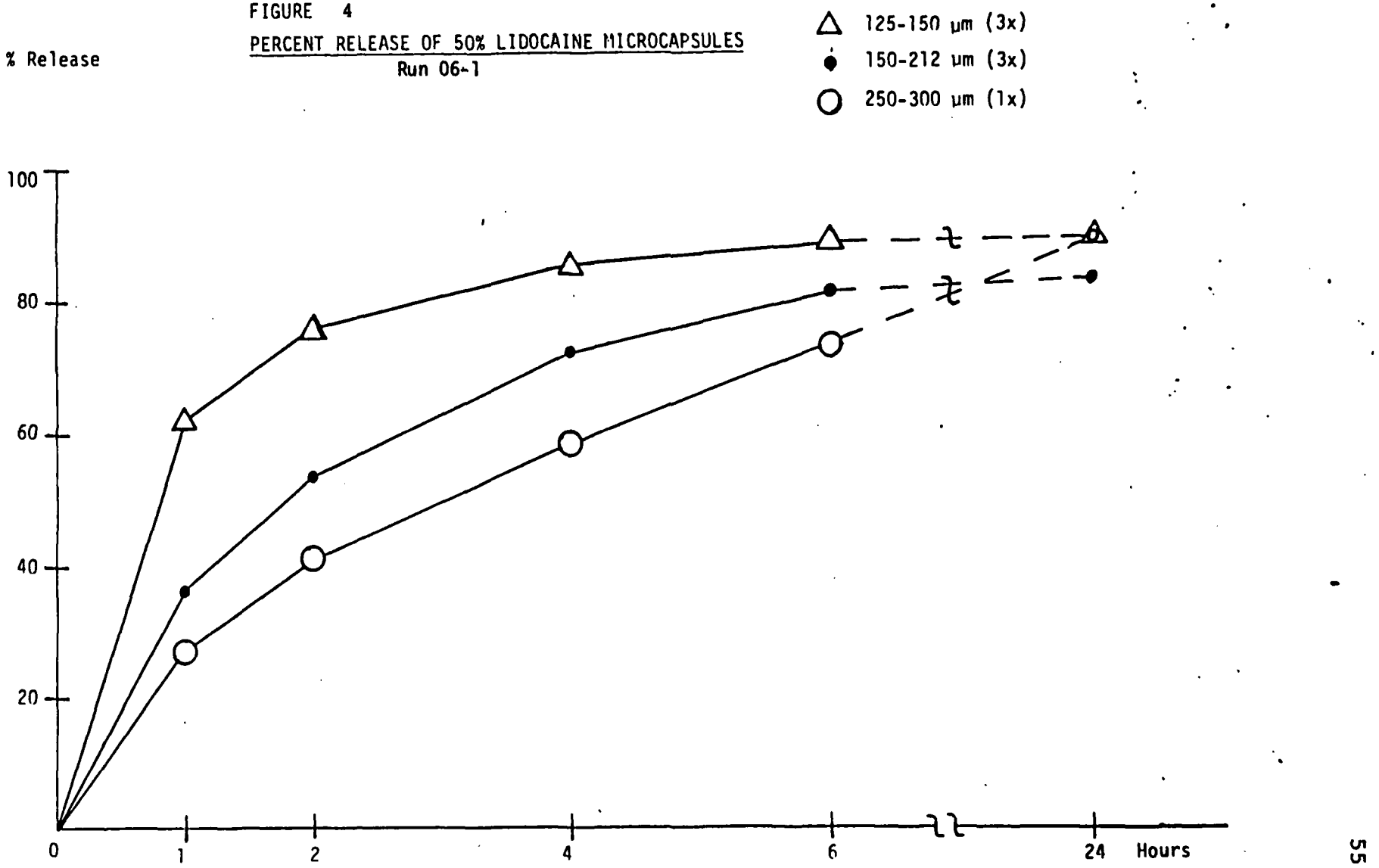
b) Reproducibility of In Vitro Assay (50% Coating)

Size Range (μm)	Test	% Release at Hours 1	2	4	6	24
125-150	1	68.8	78.8	84.2	87.4	89.7
	2	53.7	72.7	84.1	86.7	86.7
	3	63.6	77.3	88.9	91.5	94.7
	Mean	62.0	76.3	85.7	88.5	90.3
	± S. D.	7.7	3.2	2.7	2.6	4.1
150-212	1	43.7	56.2	71.6	79.2	83.7
	2	28.9	50.7	71.6	78.6	81.4
	3	36.2	53.9	73.6	86.7	84.8
	Mean	36.3	53.6	72.3	81.5	83.3
	± S. D.	7.4	2.8	1.2	4.5	1.7

FIGURE 4

PERCENT RELEASE OF 50% LIDOCAINE MICROCAPSULES

Run 06-1



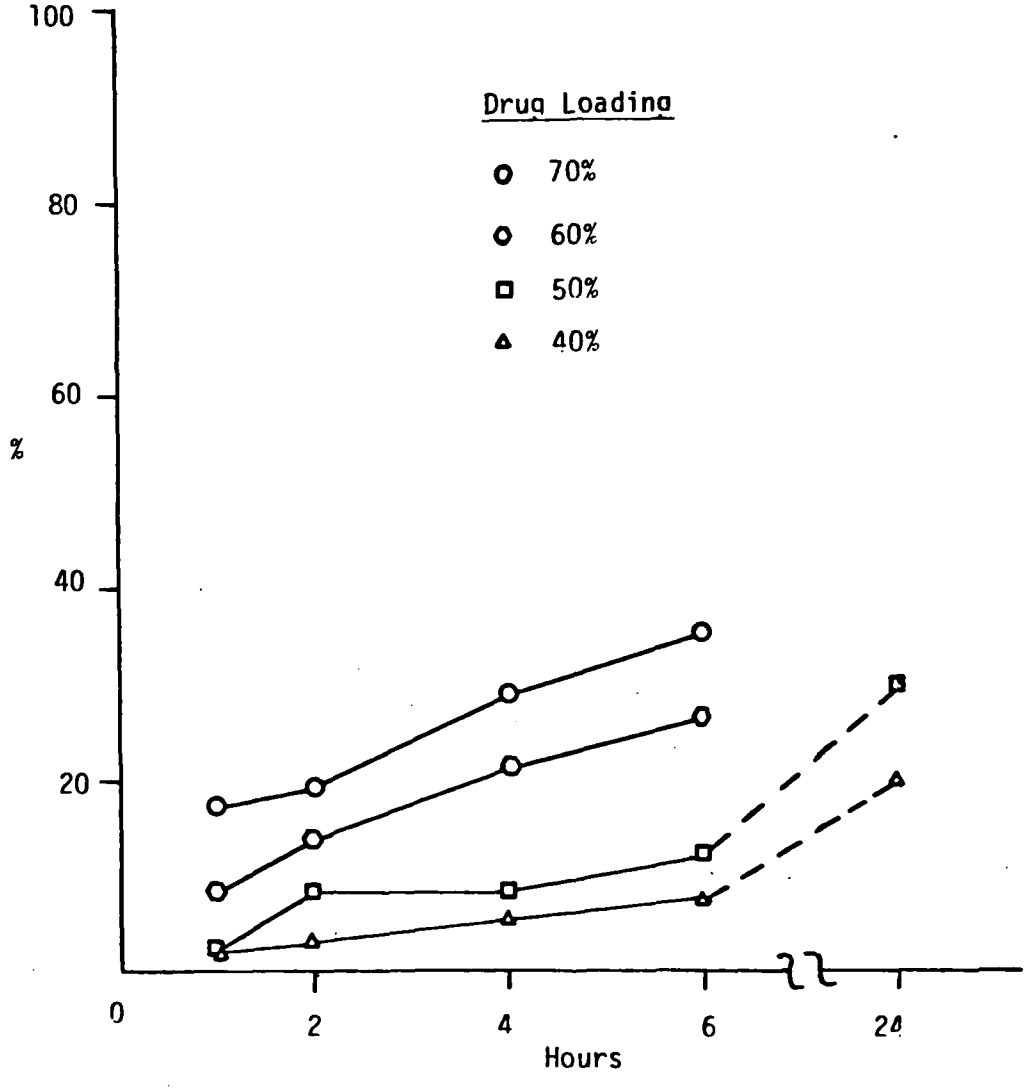


FIGURE 5 PERCENT RELEASE OF 212-300 μm MICROCAPSULES CONTAINING VARIOUS AMOUNTS OF ETIDOCAINE (Run 06-2)

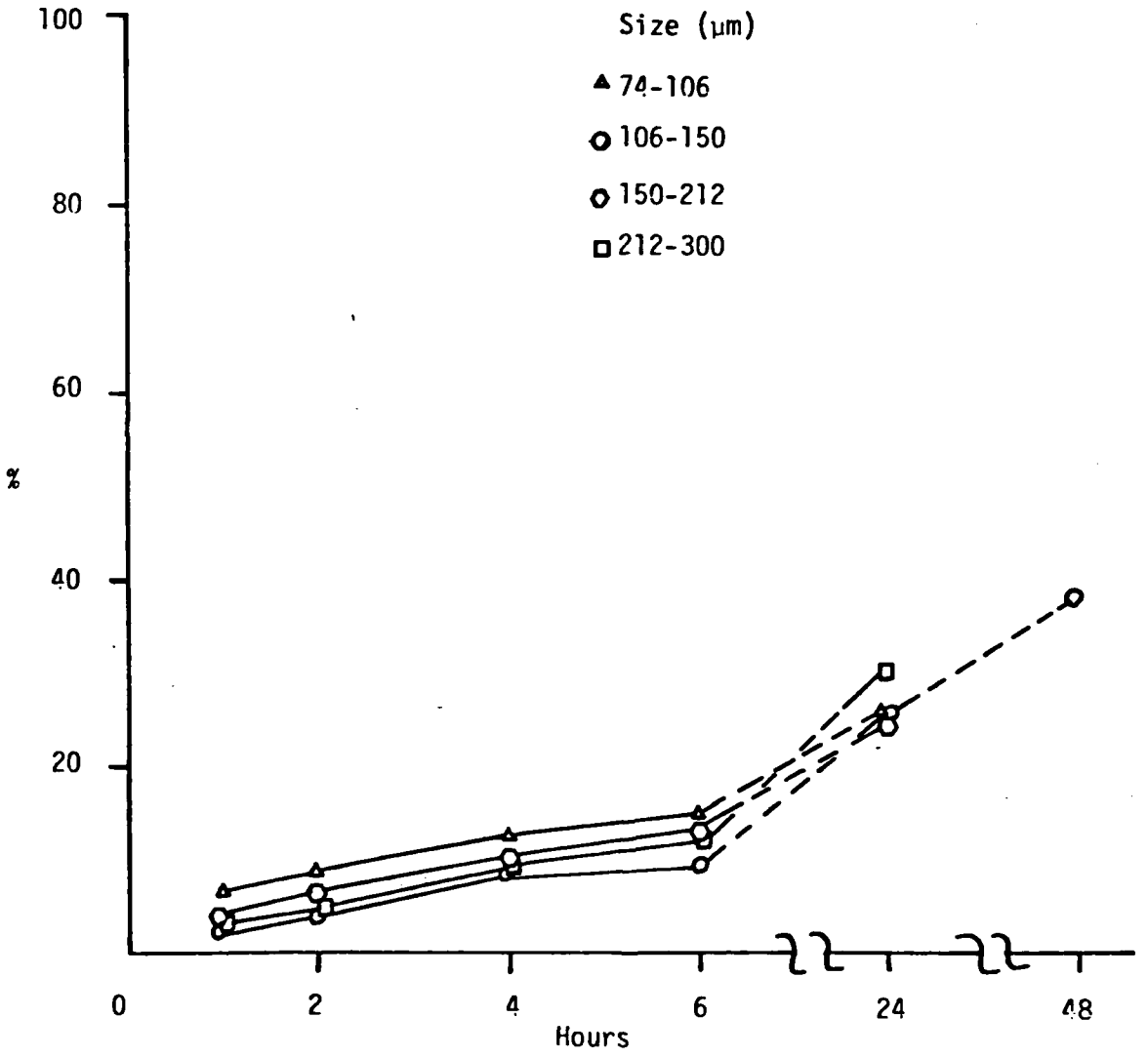


FIGURE 6 PERCENT RELEASE OF 50% ETIDOCAINE MICROCAPSULES OF VARIOUS SIEVE SIZES (Run 06-2)

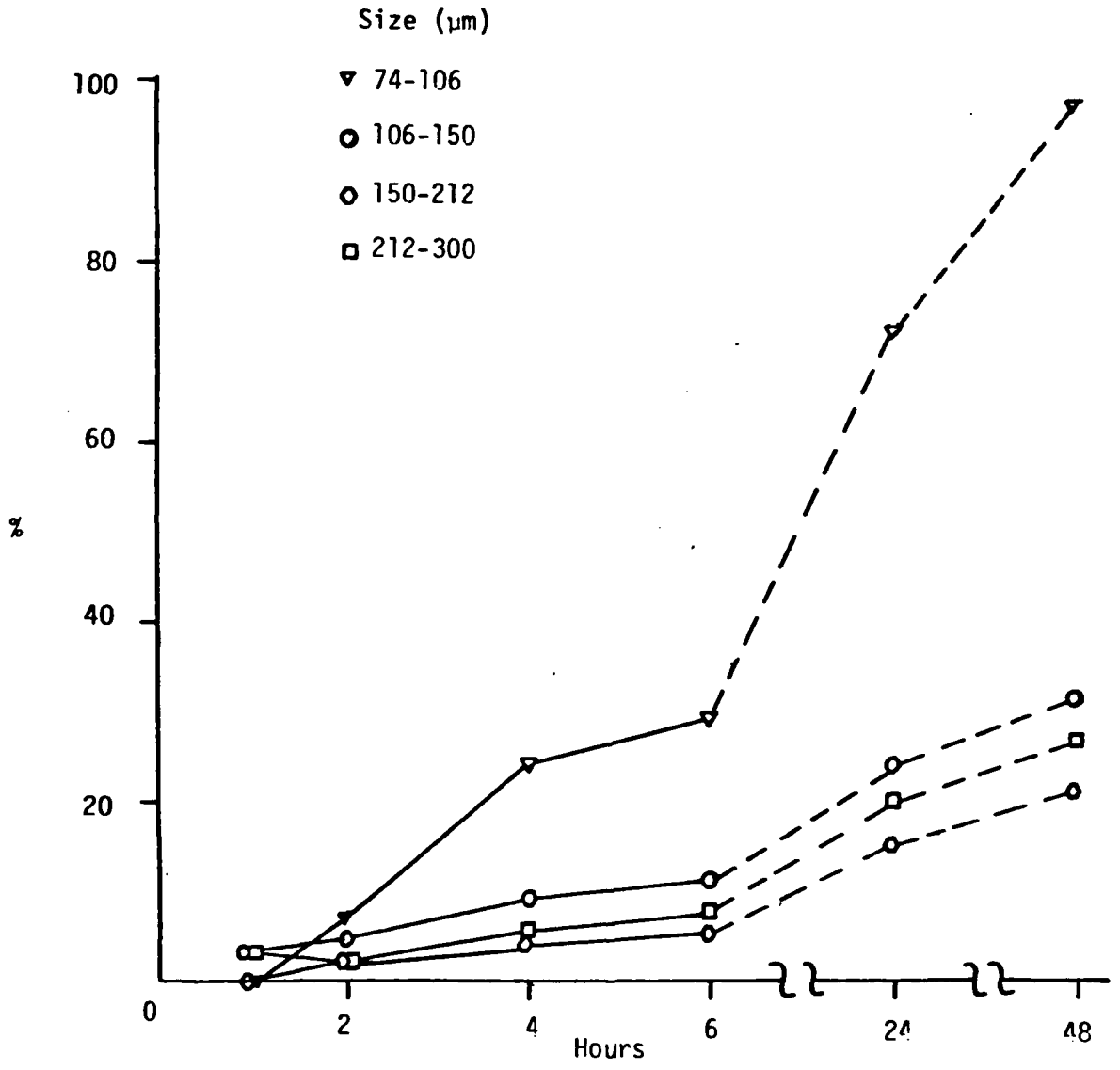


FIGURE 7 PERCENT RELEASE OF 40% ETIDOCAINE MICROCAPSULES OF VARIOUS SIZE RANGES (Run 06-2)

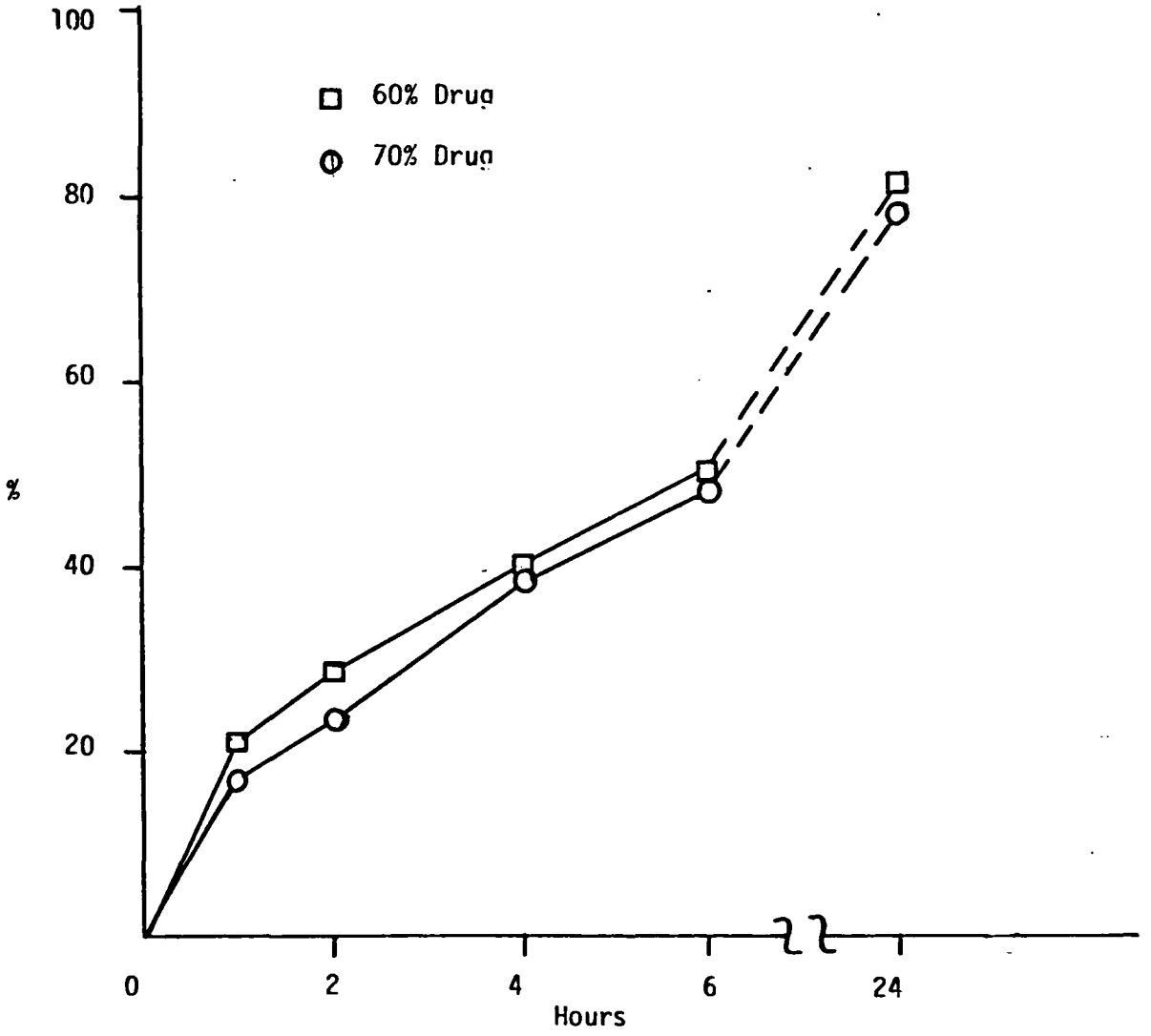


FIGURE 8 PERCENT RELEASE OF 106 to 74 μm ETIDOCAINE MICROCAPSULES OF VARIOUS PERCENT LOADINGS (Run 06-2)

TABLE 21

IN VITRO RELEASE OF

BUPIVACAINE MICROCAPSULES

(Run 06-3)

% Drug	Size Range (μm)		% Release 1 Hr.	2 Hr.	4 Hr.	6 Hr.	24 Hr.	78 Hr.
50%	150-212		11.8	13.7	16.0	17.8	23.7	35.2
	106-150		55.3	71.2	81.7	88.8	97.2	109.4
	75-106	*	36.5	41.3	45.9	49.3	57.5	64.8
60%	150-212		10.2	12.1	14.9	17.7	25.9	41.3
	106-150		44.6	50.7	58.2	63.8	78.8	92.0
	75-106	*	87.5	88.2	89.5	90.6	90.2	90.3
70%	212-250		19.7	26.7	37.7	46.5	77.2	84.4
	150-212		19.5	27.5	40.7	51.7	79.1	80.9
	106-150	*	54.6	69.5	84.5	89.5	90.5	90.4
	75-106	*	56.1	67.0	77.4	84.1	92.0	96.6
	38-75	*	54.2	65.9	78.7	86.0	96.0	95.6
80%	150-212		27.1	38.2	56.1	69.0	84.7	86.9
	106-150		49.0	67.1	82.3	86.9	96.0	95.8
	75-106		98.0	102.0	103.9	103.5	103.5	103.4

* Diffusion data based on microcapsule assay value.
Other data based on coating level calculated value.

propose to coat with 25-30% polymer. The data of the 70% drug microcapsules of 150-212 micron size is shown in Figure 9 . From the data of Table 21 a broad size distribution might be used in the anesthetic product.

4. Lidocaine (Base) Release Studies (Run 11-1)

In the analysis of the drug content of lidocaine (base) microcapsules, it was apparent that the 30% nominal coating was actually very different for the different size microcapsules. The smallest microcapsules tested (74-105 micron) had only 10% polymer coating. In accordance with this coating level, they released their drug very rapidly (Figure 10). Conversely, the largest microcapsules must contain more than 30% polymer, and they release their drug more slowly.

Since release is measured into a phosphate buffer at pH 7.4 and a similar buffer was used for the previous lidocaine-HCl diffusion medium, the slow rate of drug release from the 212-300 micron and 300-425 micron microcapsules should occur in vivo. The drug release from 6 to 24 hours for each of these samples was most encouraging. It was also encouraging that about 50% of the bed material was in this size range.

5. Etidocaine-HCl Release Studies (Run 11-2)

The drug release from the etidocaine-HCl microcapsules was relatively similar to the previous encapsulation of this drug. However, the run was stopped after rapidly achieving the 30% coating level. Thus a large, useful sample was obtained. The drug release for 20 and 30% coated microcapsules is shown in Figures 11 and 12. The results show little change of drug coating on size and also little change of release rate with different size microcapsules. Again, the release between 8 and 24 hours is encouraging for the 30% coated microcapsules.

6. Bupivacaine-HCl Release Studies (Run 11-3)

The release of bupivacaine from microcapsules of Run 11-3 indicates that the smallest particles were poorly coated (Figures 13 and 14, low polymer content and fast release). However, microcapsules of 150 micron and larger are similar in percent coating and in release behavior. Both 20 and 30% nominal coating levels yielded microcapsules having potentially useful drug release profiles.

7. Etidocaine-HCl Release Studies (Run 11-4)

Selected microcapsules from Run 11-4 were tested for drug release. The addition of polymer to the core of the microcapsule did not significantly change the drug release profile.

FIGURE 9 RELEASE OF BUPIVACAINE MICROCAPSULES

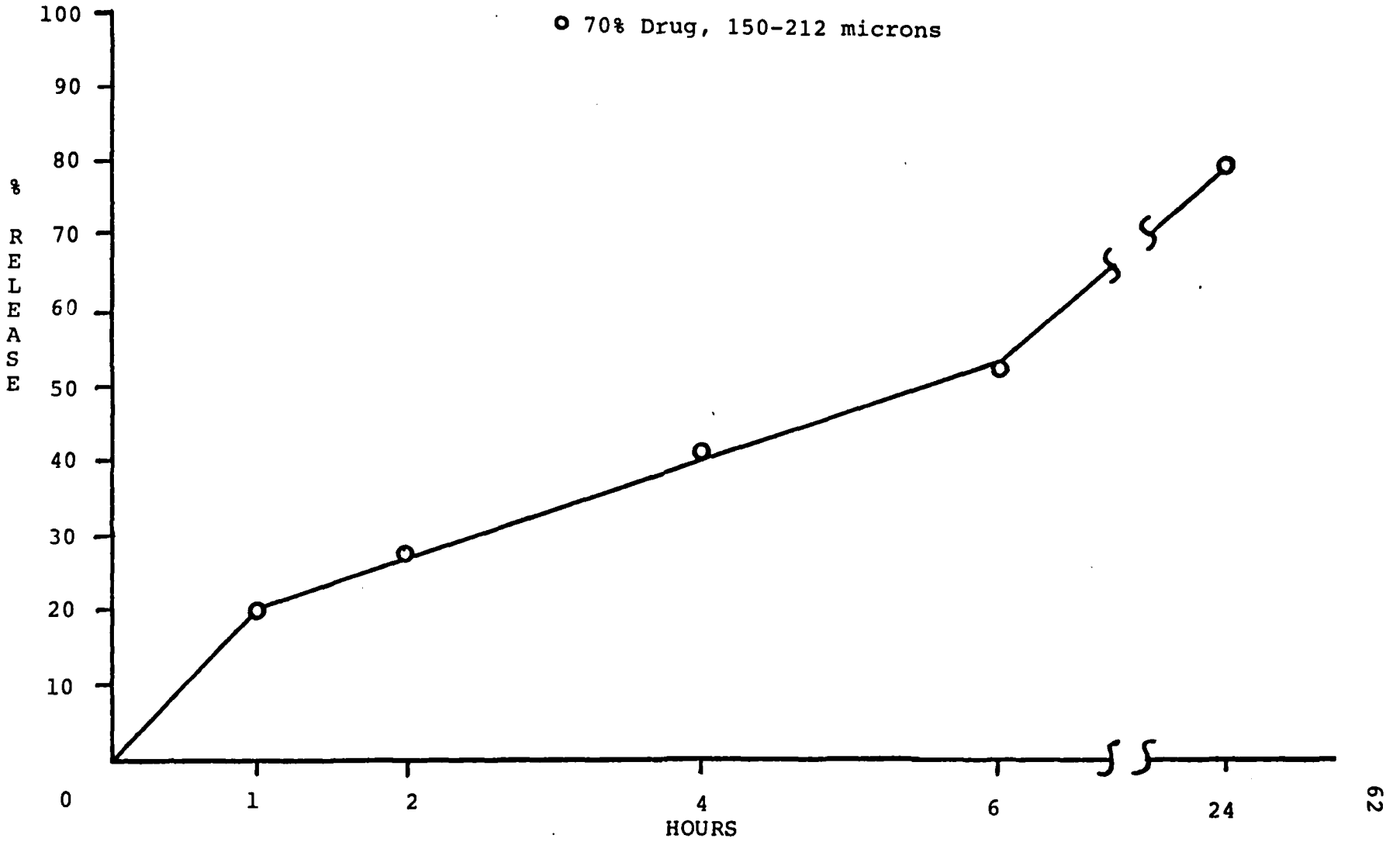
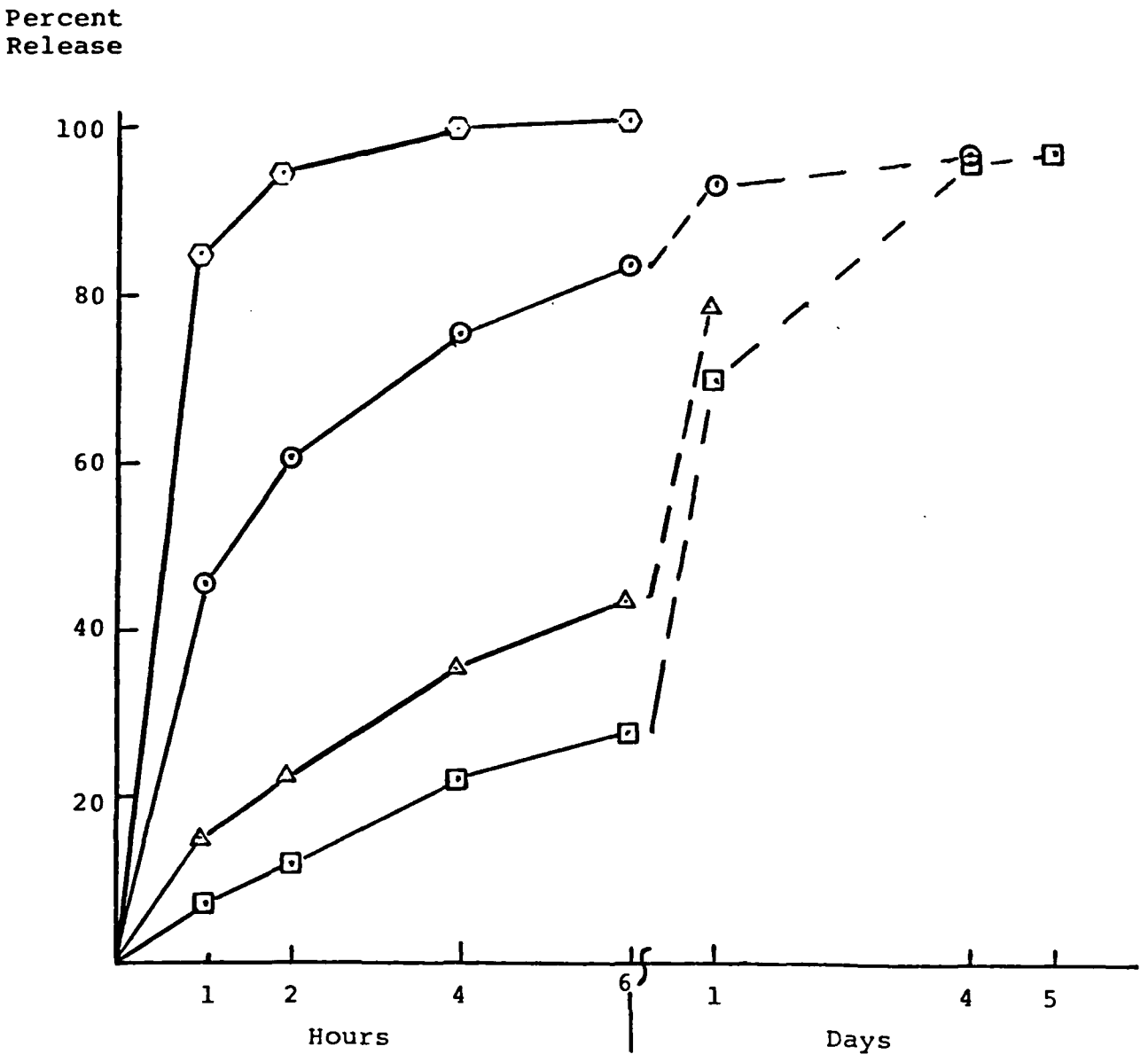


FIGURE 10

IN VITRO RELEASE OF LIDOCAINE BASE

FROM MICROCAPSULES (11-1-30)

	Size (μm)	Assay Drug %	% of Bed
⬡	74-106	90	5
○	150-212	75	8
△	212-300	72	23
□	300-425	68	28



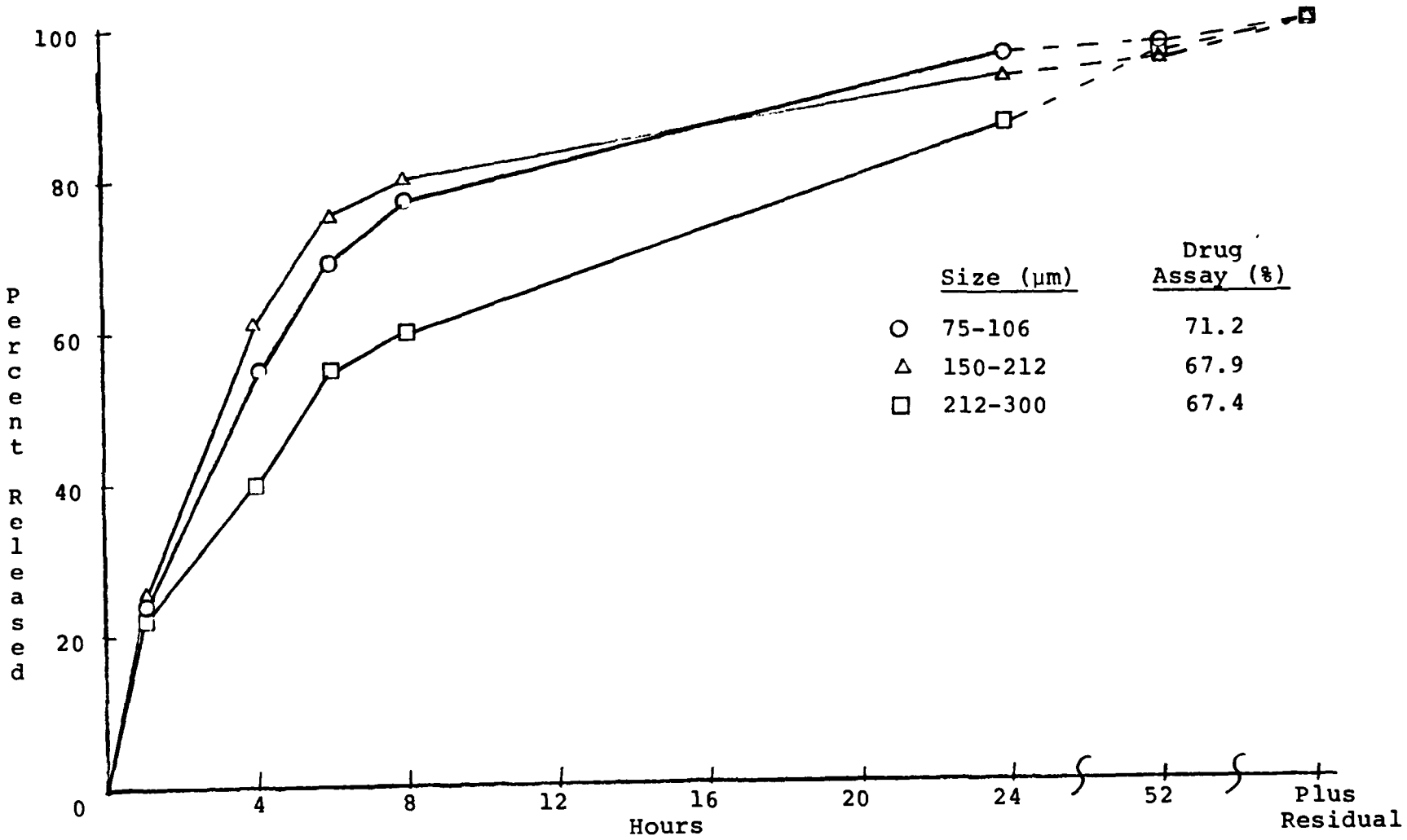


Figure 11 Cumulative Release of Etidocaine·HCl from 11-2-20 Microcapsules (Corrected to 100% from higher values)

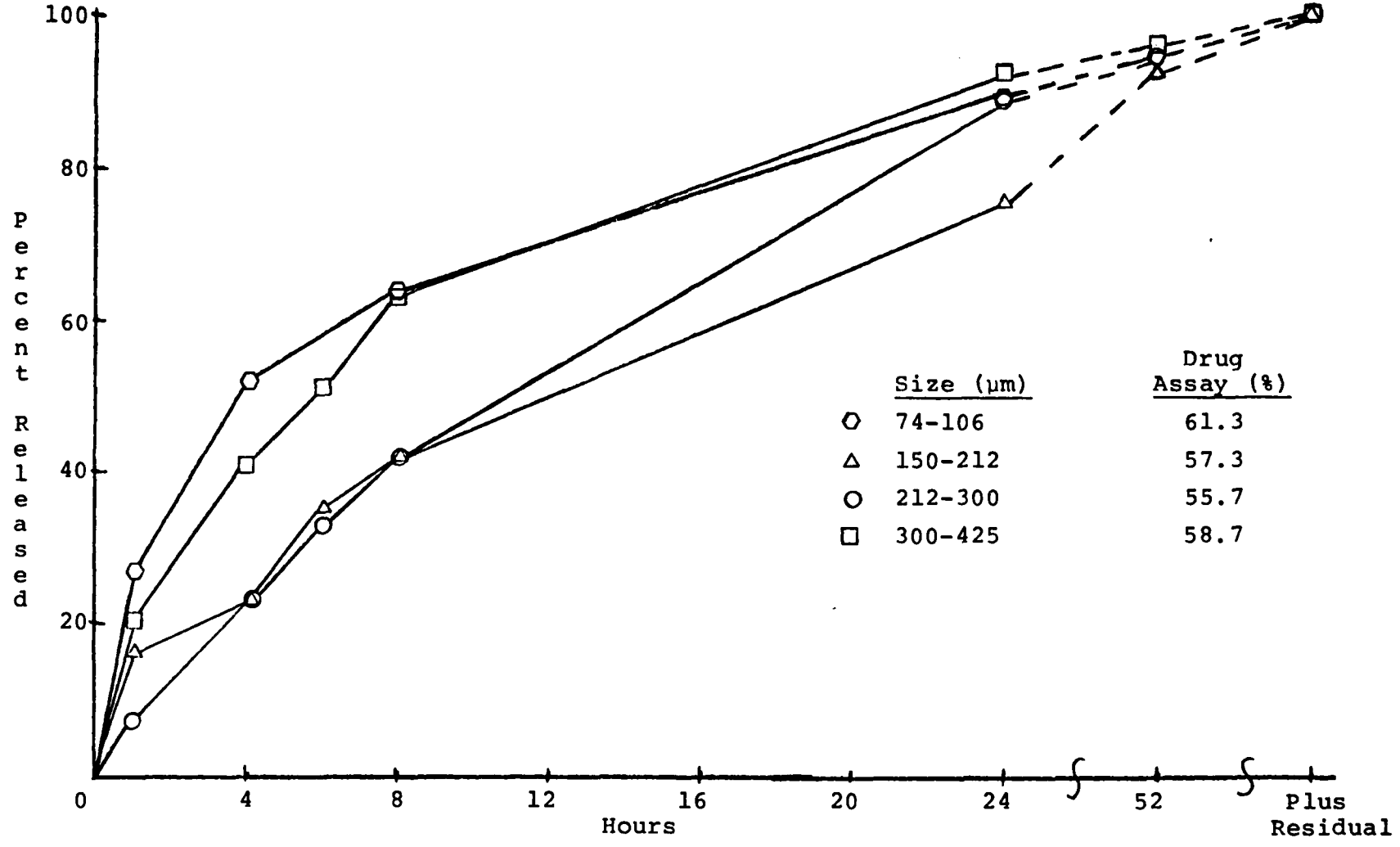


Figure 12 Cumulative Release of Etidocaine·HCl from 11-2-30 Microcapsules (Values decreased to 100% totals)

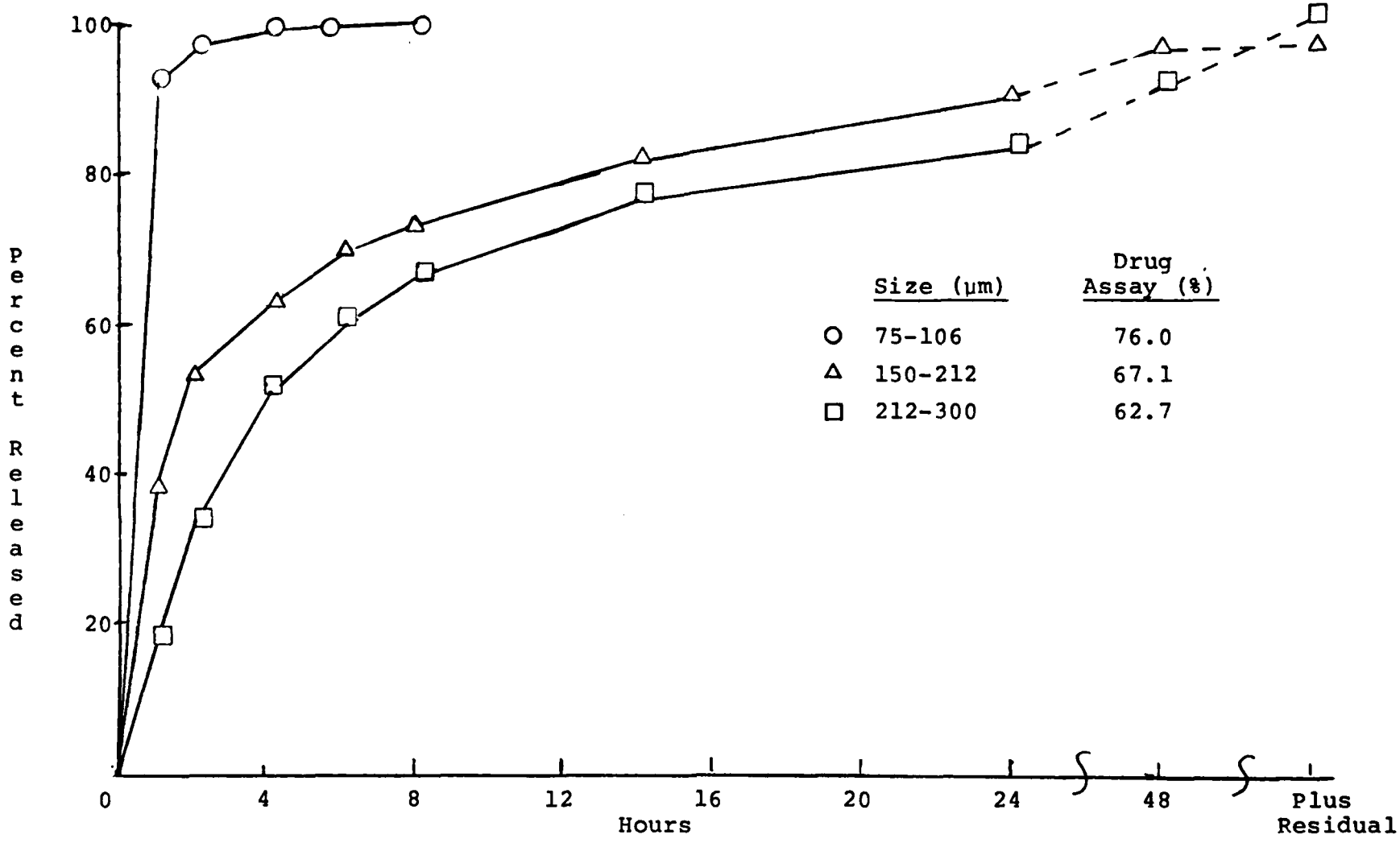


Figure 13 Cumulative Release of Bupivacaine·HCl from 11-3-20 Microcapsules

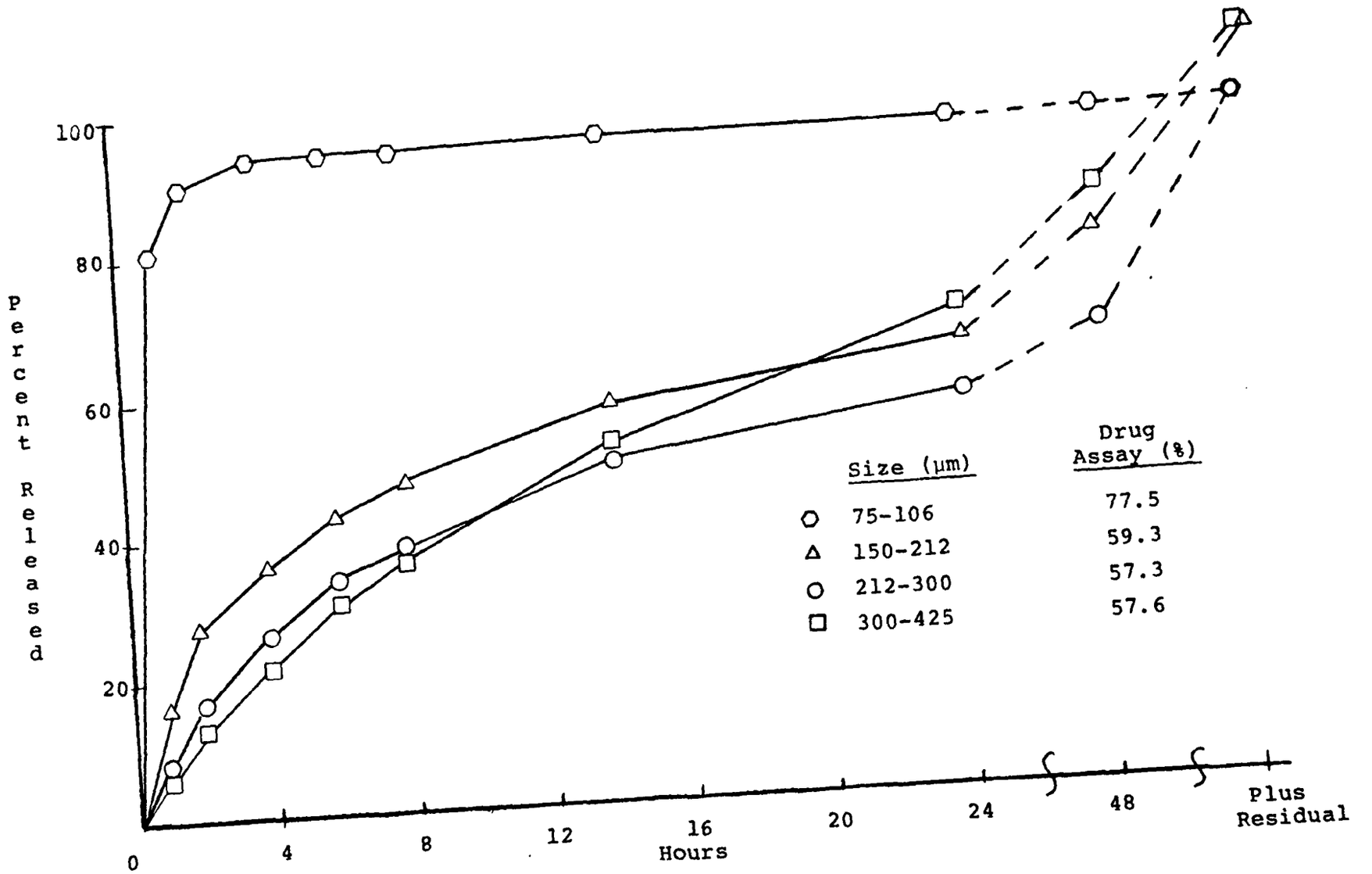


Figure 14 Cumulative Release of Bupivacaine·HCl from 11-3-30 Microcapsules

The data are shown in Figures 15-17. In general, smaller microcapsules and capsules with lower polymer coatings release drug more rapidly.

8. Multi-Point Microcapsule Release Studies

For convenience, data points are normally taken at 1, 2, 4, 6, and 24 hours. However, for the release of drug from selected microcapsules a schedule was devised which allowed more data points to be taken. Separate sets of microcapsules were started in the early morning and late afternoon. Solutions were also measured and changed once in the evening. The data of selected microcapsules are shown in Figures 18 and 19. With this number of points, significant release rate data can also be plotted (Figures 20-22). These curves are the derivative of the cumulative data which has been plotted above. The release rate curves show a release equivalent to an infusion process.

The data are generally similar to that obtained previously. The microcapsules varied in age from 2 to 12 months. All the samples had been stored in sealed vials at room temperature.

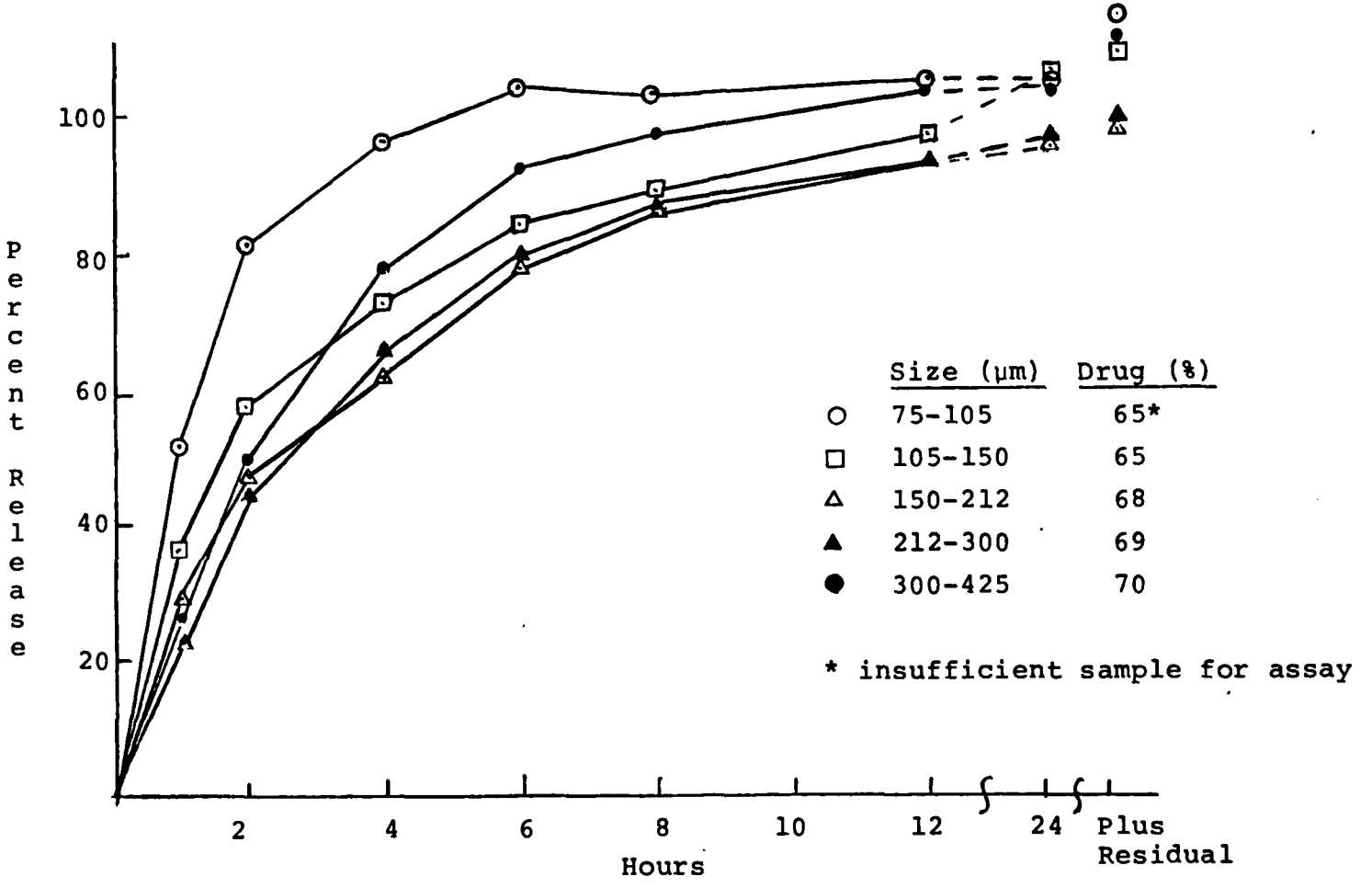


Figure 15 Etidocaine-HCl Release at 10% Coating Level (11-4-10)

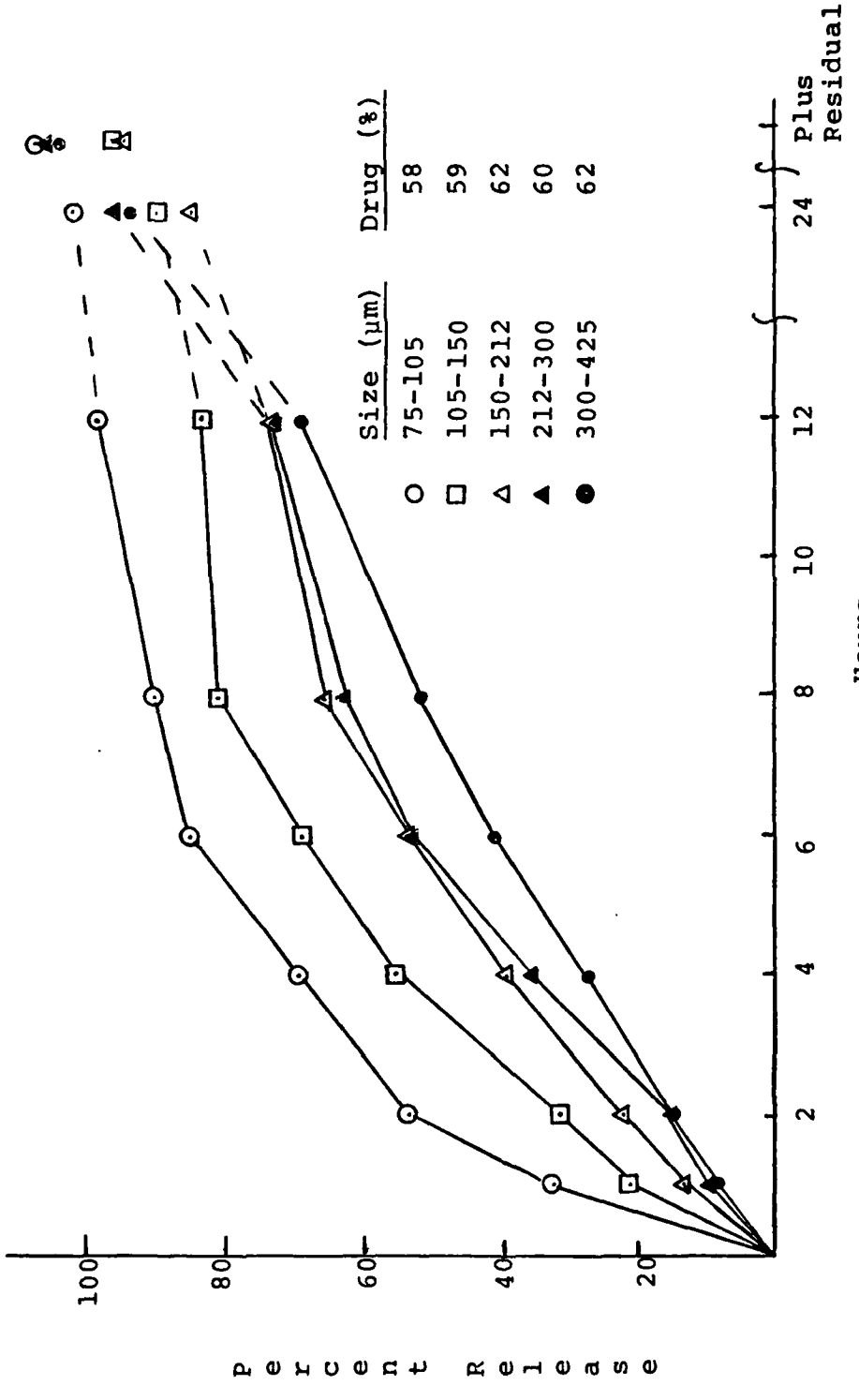


Figure 16 Etidocaine-HCl Release at 20% Coating Level (11-4-20)

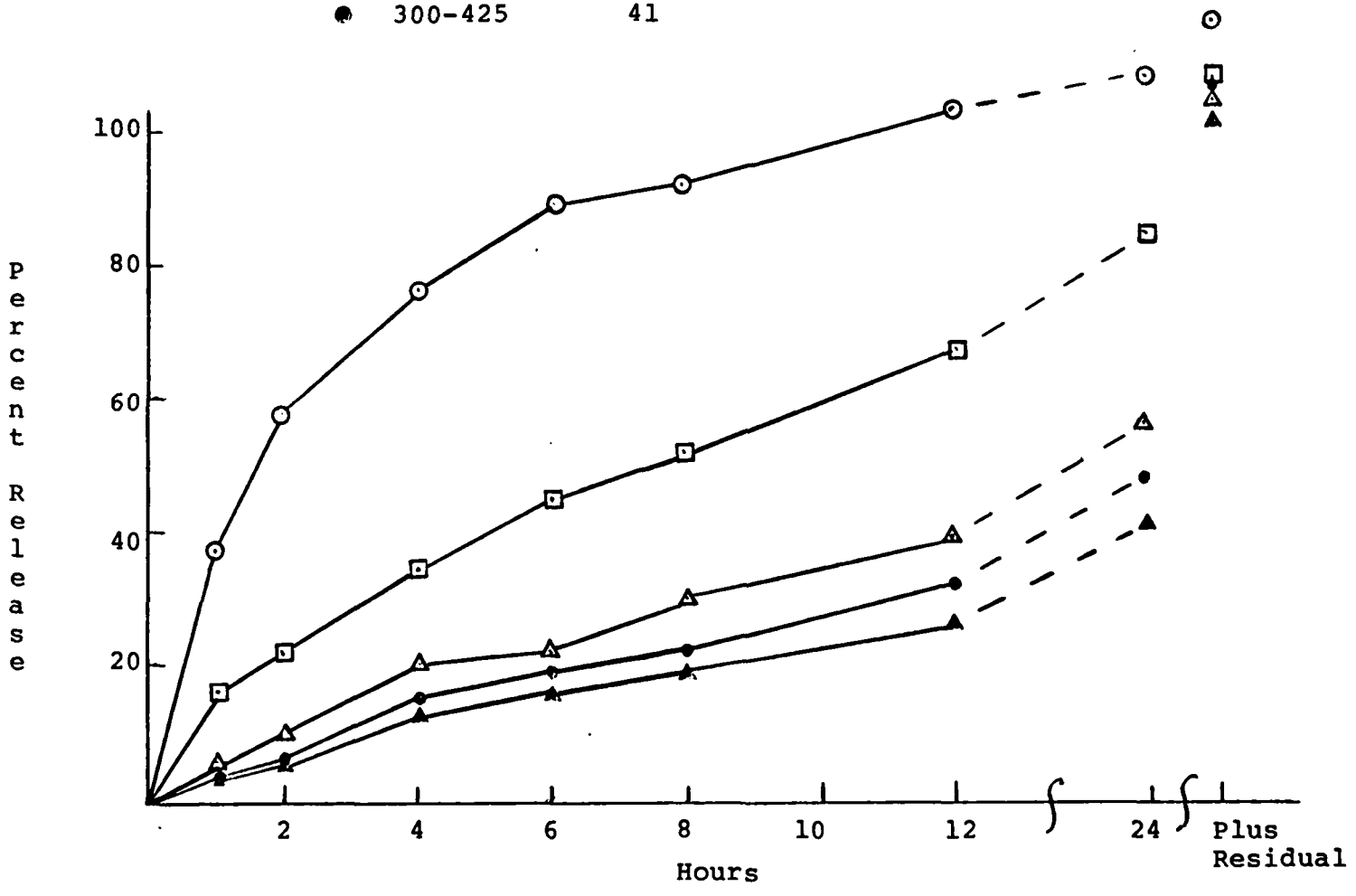


Figure 17 Etidocaine-HCl Release at 30% Coating Level (11-4-30)

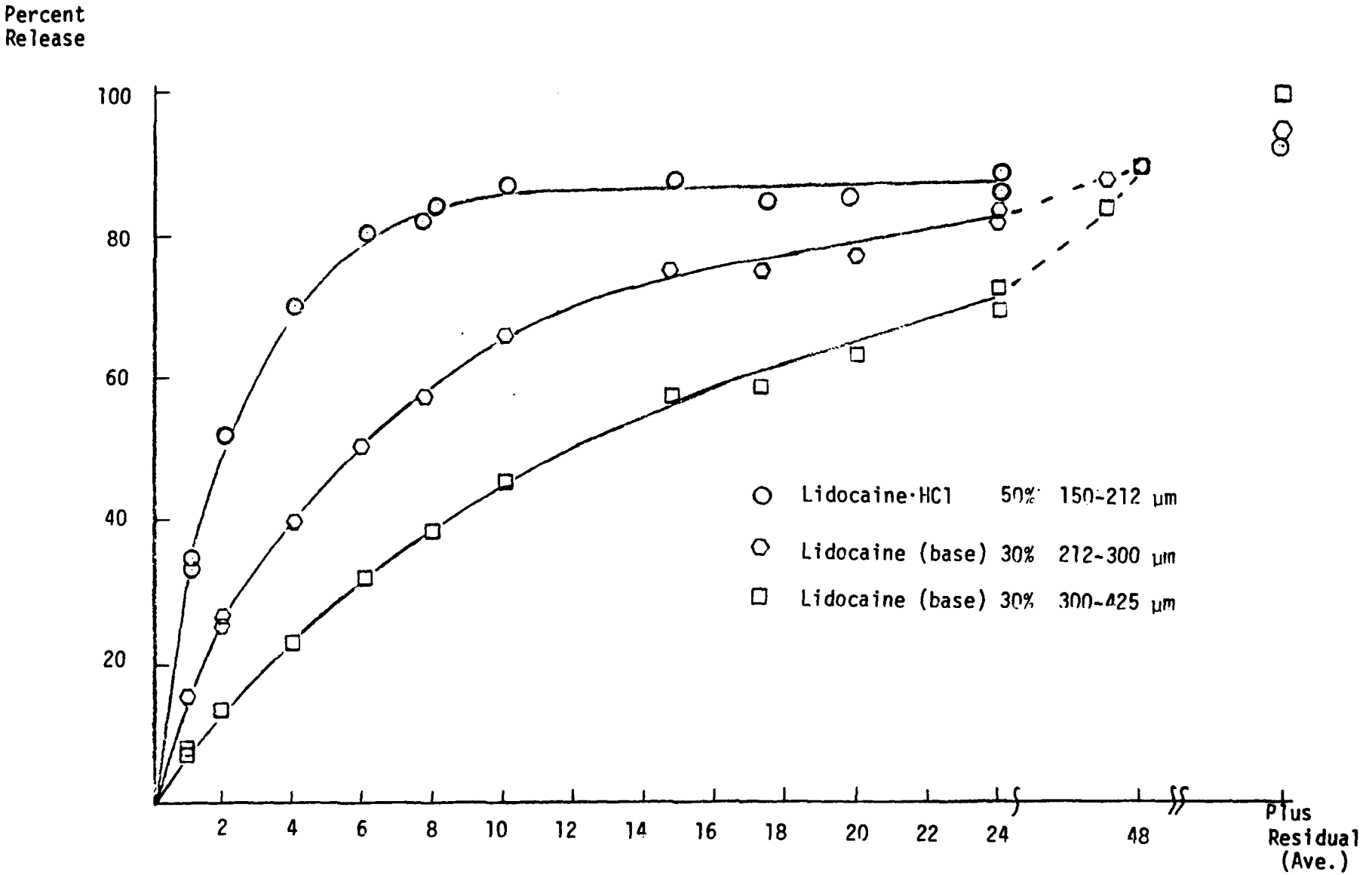


Figure 18 Cumulative Release of Lidocaine from Selected Microcapsules

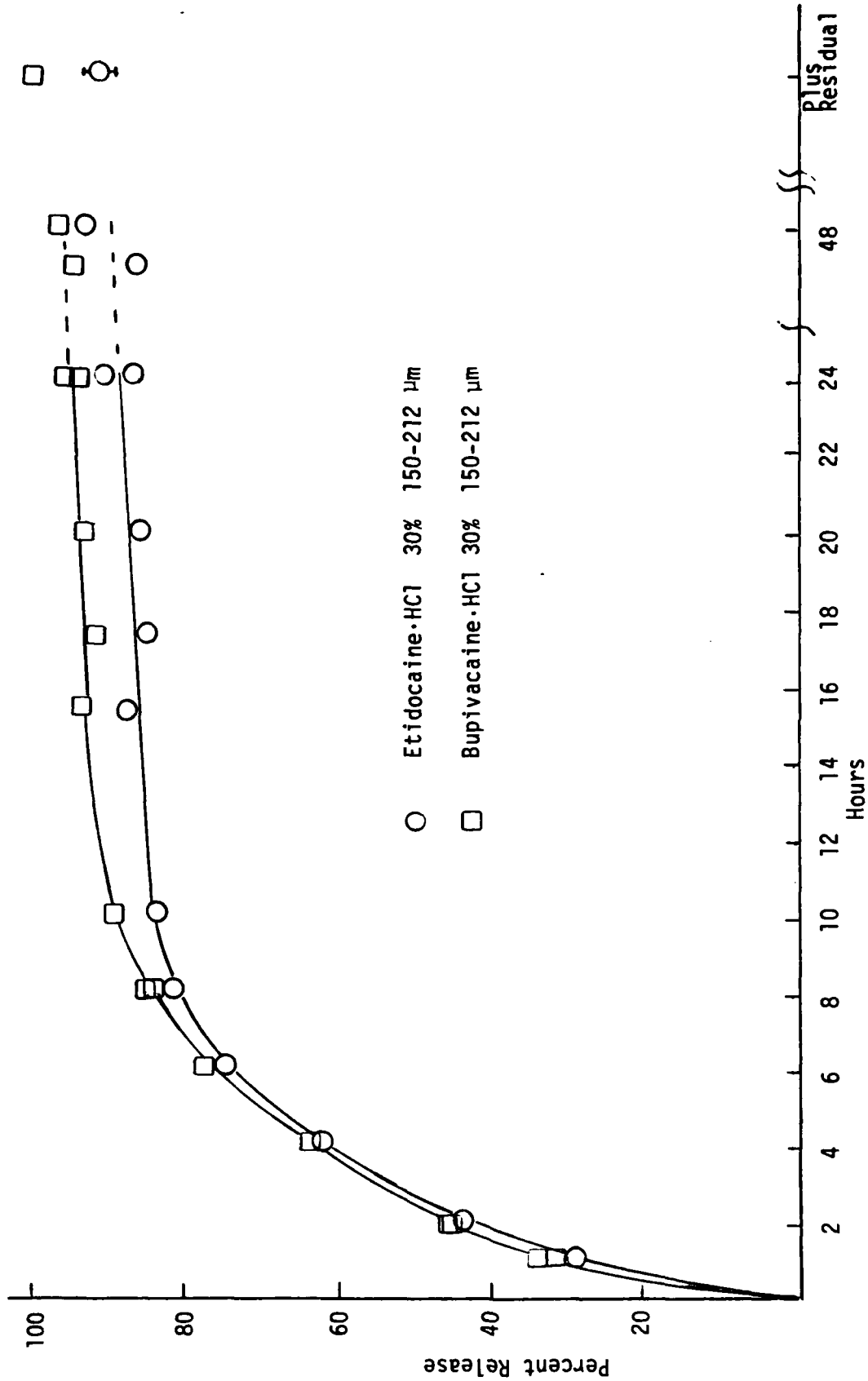


Figure 19 Cumulative Release of Etidocaine and Bupivacaine from Selected Microcapsules

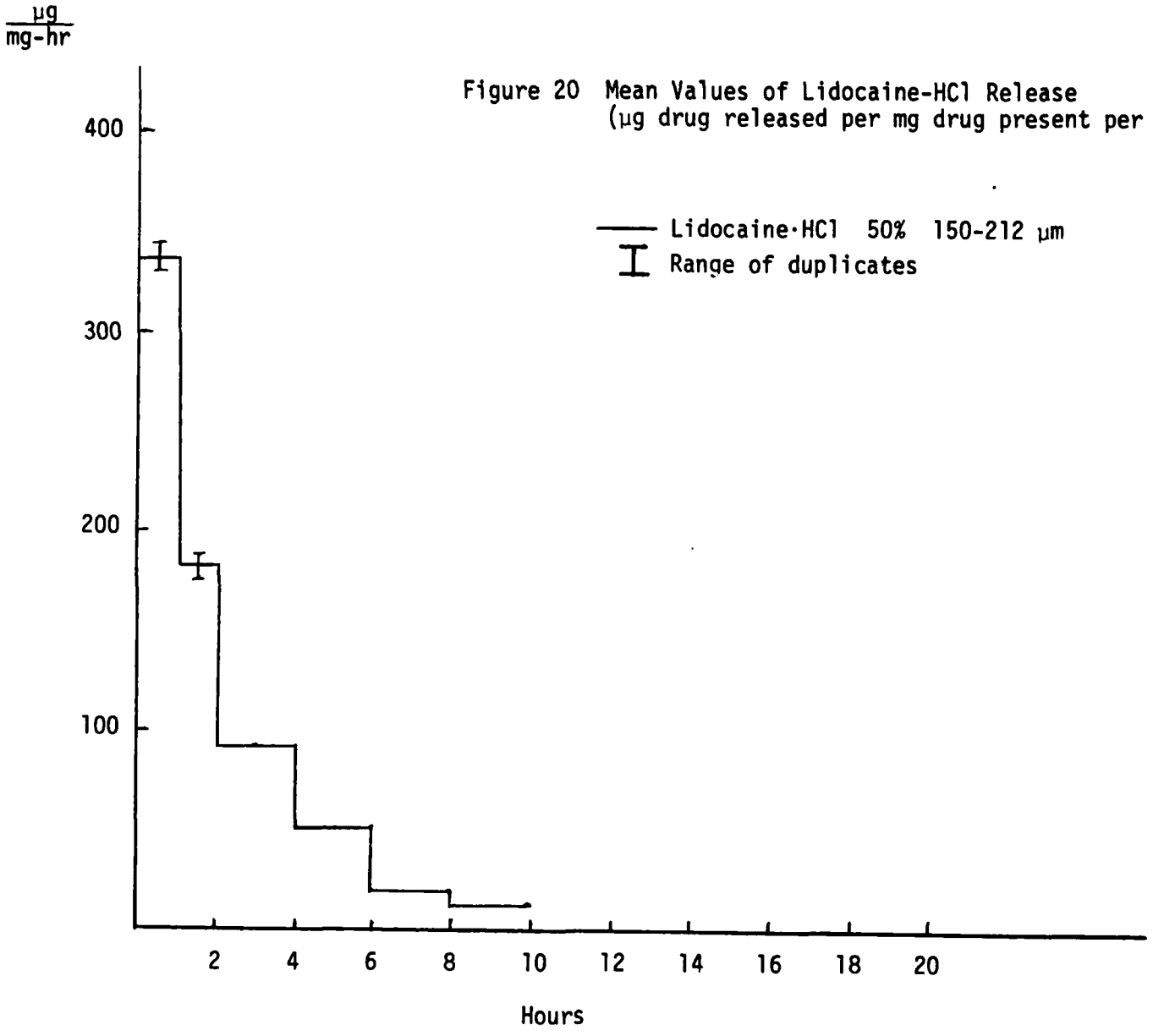


Figure 20 Mean Values of Lidocaine-HCl Release
(μg drug released per mg drug present per hour)

Figure 21 Mean Values of Lidocaine (base) Release
(µg drug released per mg drug present per hour)

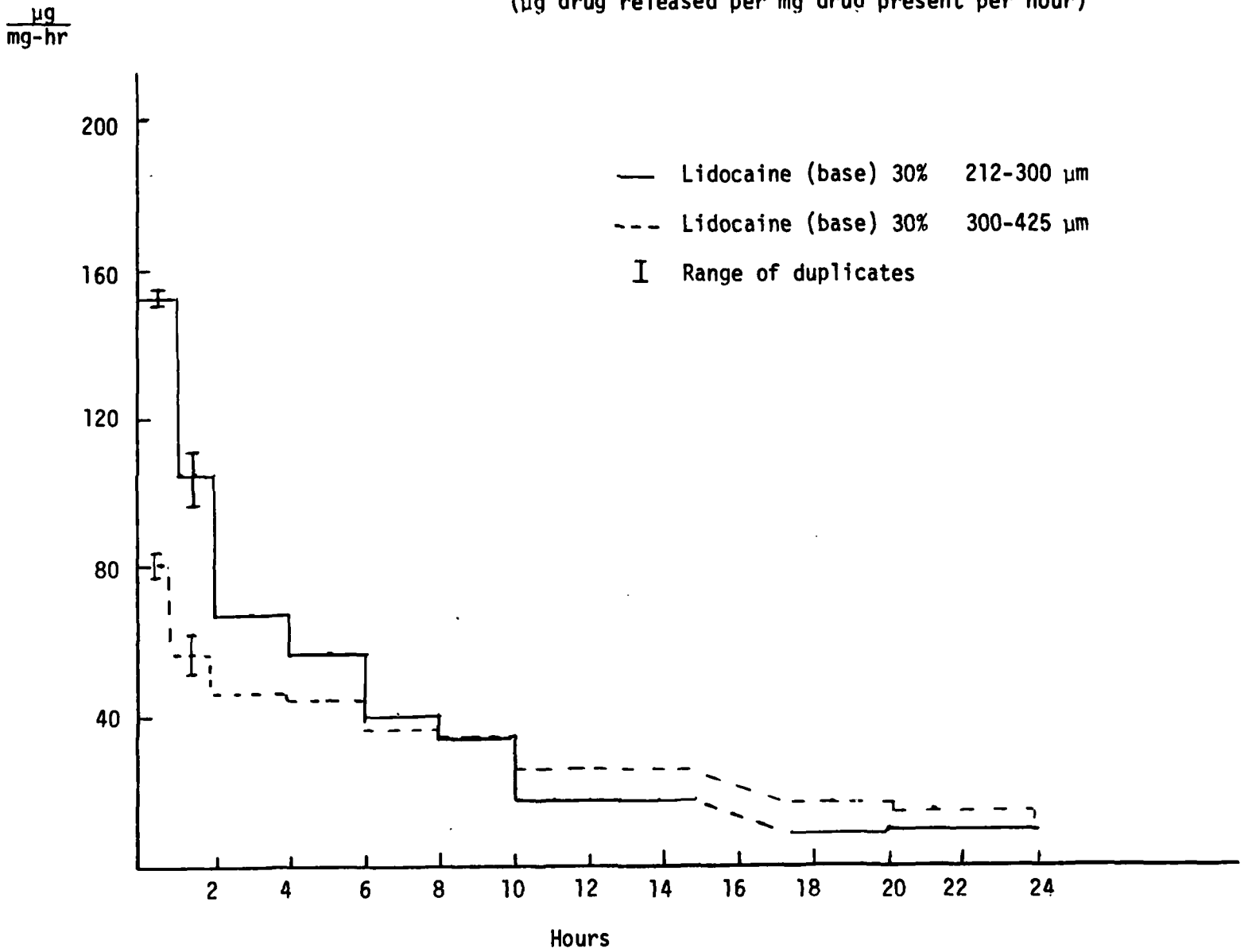
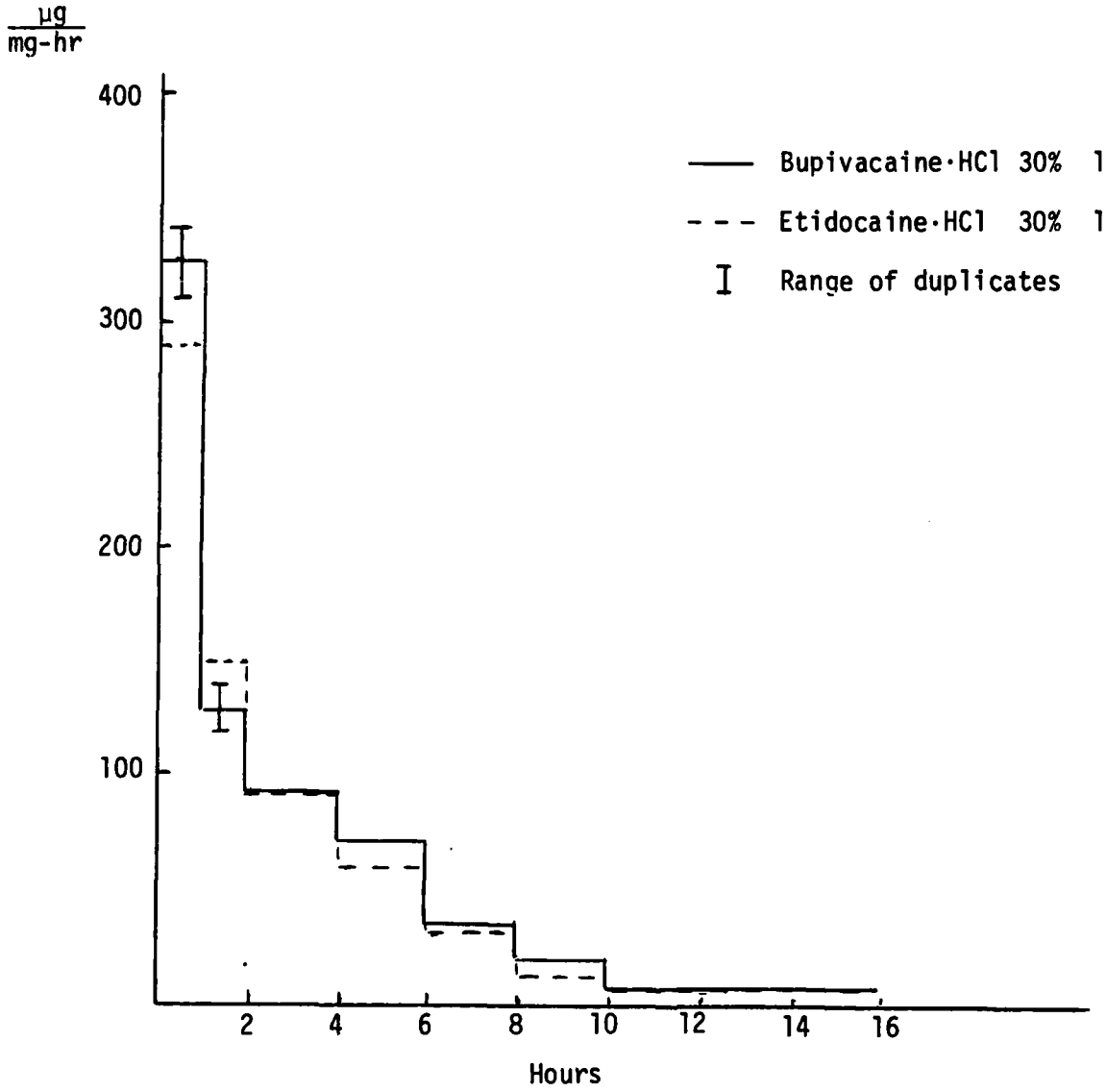


Figure 22 Mean Values of Etidocaine and Bupivacaine Release
(µg drug released per mg drug present per hour)

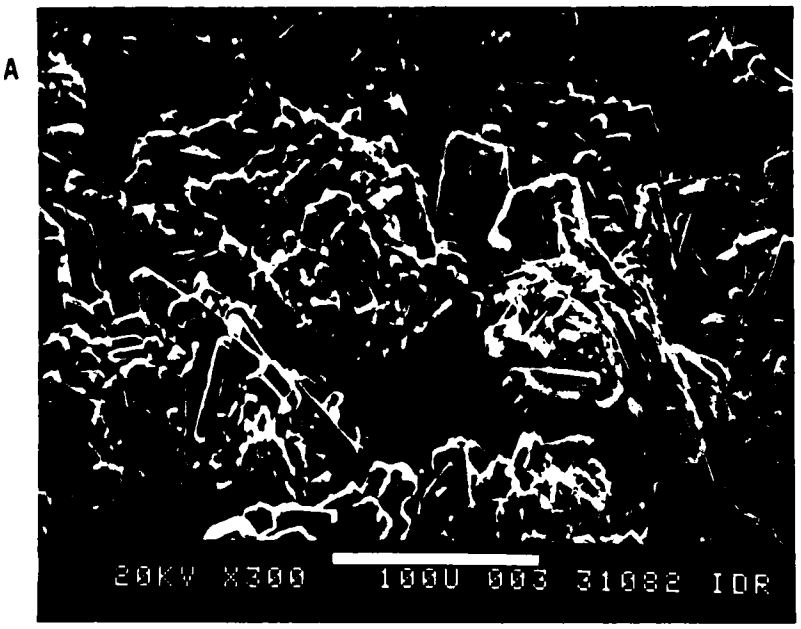


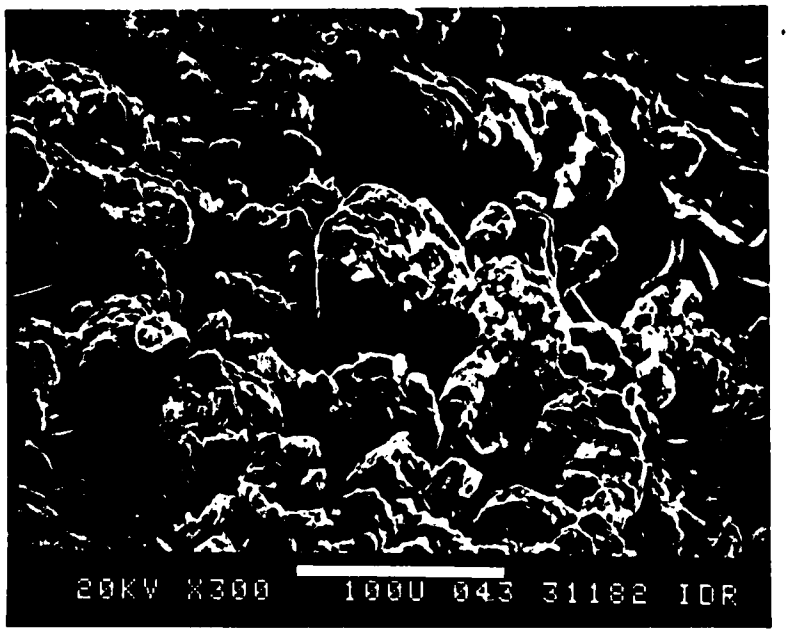
F. MICROCAPSULE MORPHOLOGY

Scanning electron micrographs (SEM) of microcapsules have been prepared by the USAIDR Biophysics Department. Scanning electron micrographs of selected starting materials and microcapsules are shown in Figures 23 and 24.

Bupivacaine-HCl (Figure 23) is representative of the needle-like crystals of the amide hydrochloride anesthetics. Small crystals are often imperfectly coated as seen in Figure 23-B. An amorphous polymer layer normally covers those microcapsules which exhibit useful drug release behavior (Run 11-3, Figure 23-C).

Lidocaine (base-USP) is composed of large crystals which apparently break up in the fluidized bed. Agglomerates were also broken up after the bed collapsed during the early stages of coating and again at 13% coating. This process generated a flowable powder which yielded well-coated microcapsules at 30% polymer loading. However, small particles continued to be present which were primarily drug crystals. Many of these particles were covering the walls of the expansion chamber and were therefore not being circulated in the fluidized bed (Figure 24).





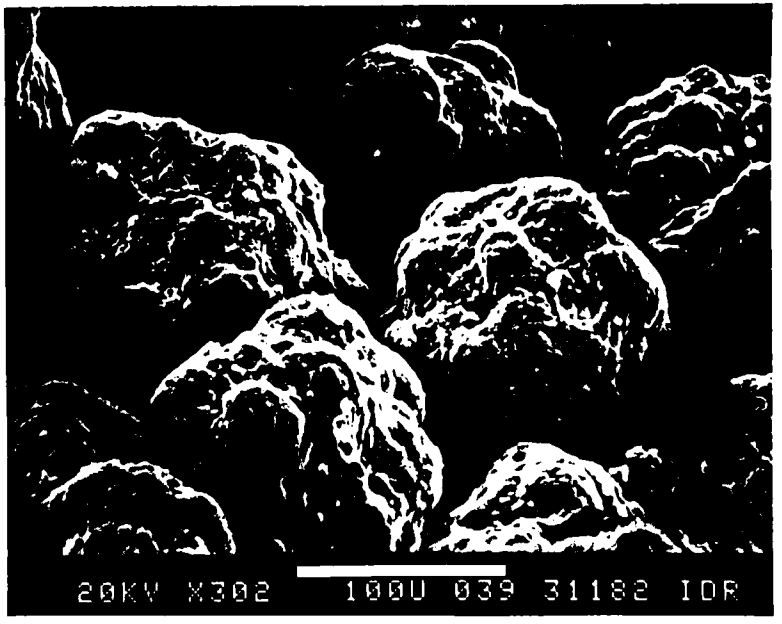
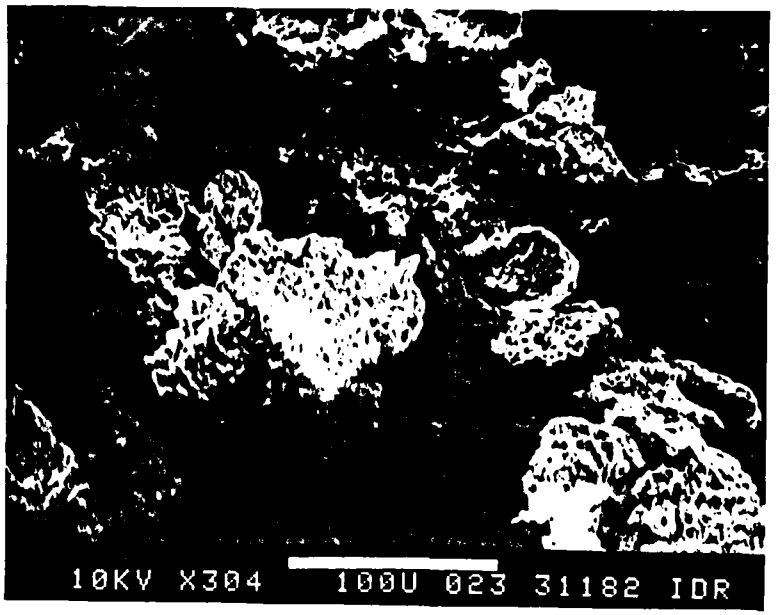


Figure 23
SEMs of Bupivacaine-HCl Microencapsulation (300x)

A) Core Material

B) 30% Coating, 75-106 micron microcapsules

C) 30% Coating, 150-212 micron microcapsules



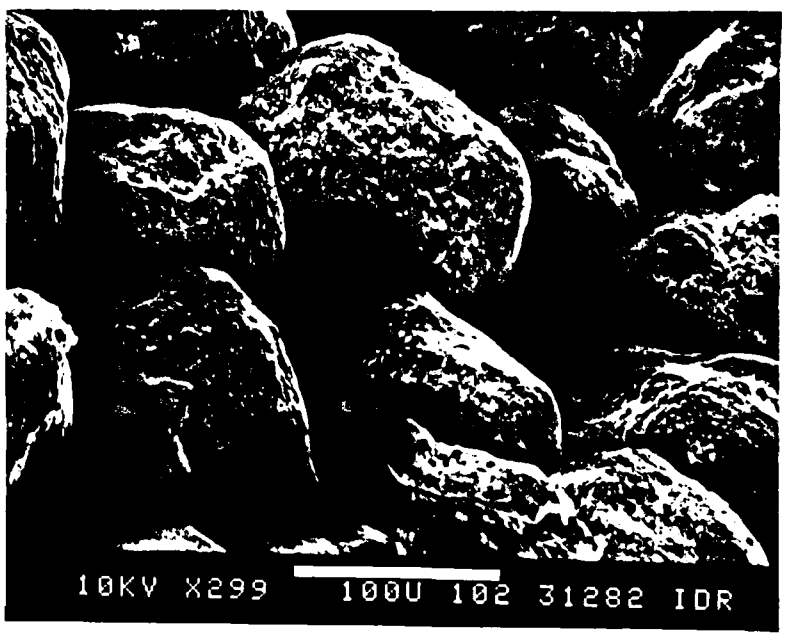


Figure 24

SEMs of Lidocaine (base) Microencapsulation (300x)

A) Core Material

B) 30% Coating, 75-106 micron microcapsules

C) 30% Coating, 150-212 micron microcapsules

G. MICROCAPSULE SURFACE DRUG CONCENTRATIONS

For quality control and correlation to drug release properties, the amount of drug on the surface of the anesthetic microcapsules might also be a useful parameter. In the coating process, a core of drug is covered with polymer. However, because of the direct correlation of drug release and water solubility of various drugs, we have postulated that much of the drug release is probably due to imperfections in the coating. These imperfections could be drug incorporated into the coating.

Fortunately, the chloride of the local anesthetic hydrochlorides can be quantitated on the surface by energy dispersive analysis of X-rays (EDAX). A local laboratory performed this analysis with an EDAX 707 and Amray 1000 SEM. Samples were coated with a thin layer of gold-palladium, since graphite coating might cause drug decomposition and loss of hydrogen chloride. Bupivacaine hydrochloride microcapsules were used as a typical anesthetic microcapsule (70% drug, 11-3-30, 150-212 micron sieve fraction). A 20 Kev beam was used. This would penetrate about 2 microns into the sample, assuming a sample density of 1.5 g/cm . The radius of the spot on the surface is approxiamtely 1 micron.

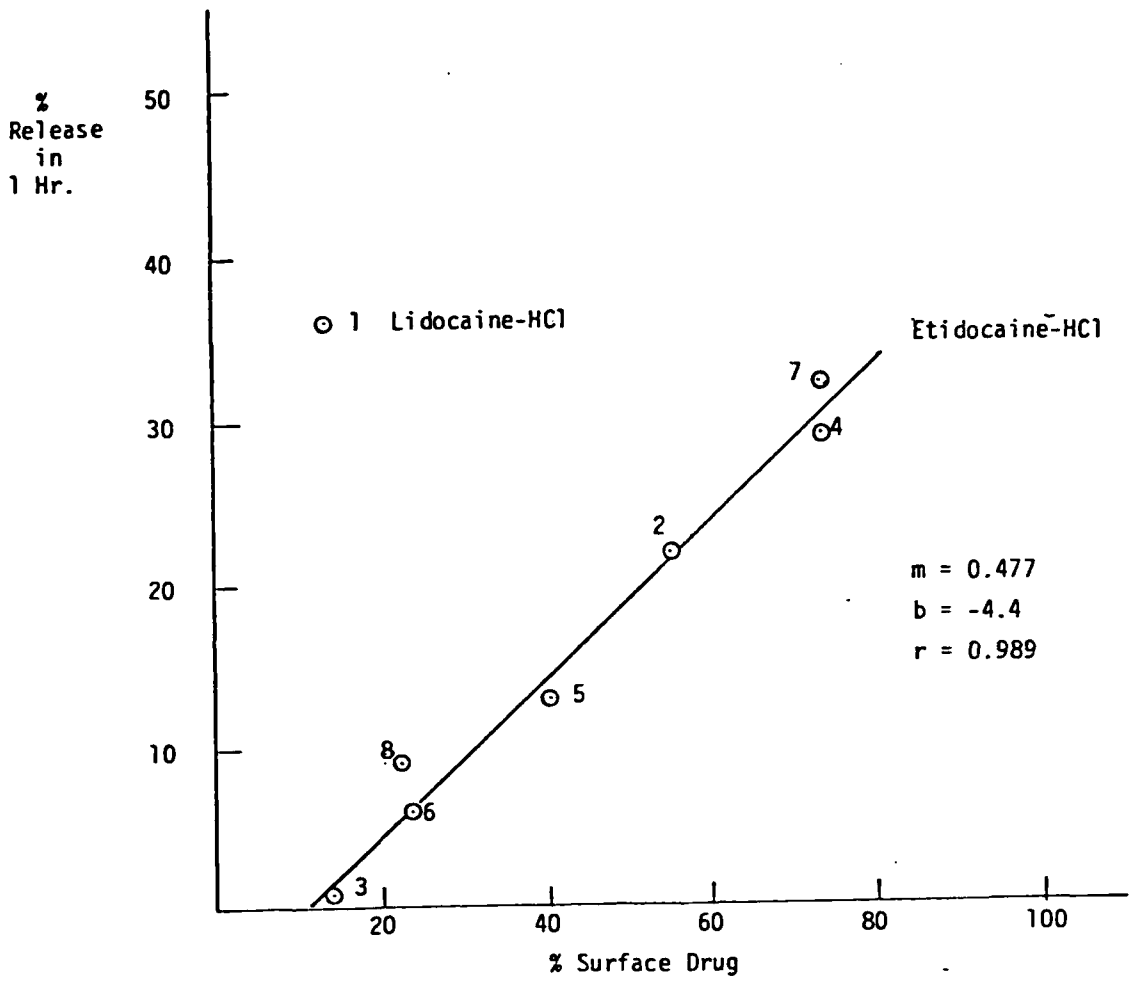
Pure bupivacaine hydrochloride had a chlorine peak of 68 c/s (counts/second) under standard conditions. Microcapsules had varying counts/second at this chloride energy peak, depending on the nature of the surface. The normal, smooth, convex surface had a count of 14.7 c/s which calculates to 22% exposed drug. However, flat or concave surfaces, where there is more structure, had counts of 33.3 c/s, corresponding to 45% exposed drug. Most of the surface was smooth.

Thus, we did find drug on the surface of the microcapsules. In a uniform appearing surface there are no obvious areas of drug segregation (solid solution or submicron domains may exist). In this surface volume there is less drug than expected from a homogeneous particle. Smooth coated surfaces contain less drug than do surfaces with more apparent structure.

In the next series of experiments a graphite paste was used which did not have to be heated, and a large area of microcapsules was scanned by the SEM for EDAX analysis.

Samples from all three microencapsulation runs of etidocaine-HCl were analyzed, as well as a single sample of microcapsules of lidocaine-HCl. Three sizes of one coating level and three coatings of one sieve fraction were analyzed for the last etidocaine microencapsulation run (see Figure 25). It was hypothesized that the initial burst of drug release would be

Figure 25 Correlation Between Surface Drug and Initial Drug Release for Microcapsules of various Amide Hydrochlorides, various Sizes, and Polymer Coating Levels



Sample	Drug-HCl	Run No.	Polymer % Coating	Size (μm)
1	Lidocaine	06-1	50	150-212
2	Etidocaine	11-2	30	106-300
3	Etidocaine	06-2	50	150-212
4	Etidocaine	11-4	10	150-212
5	Etidocaine	11-4	20	150-212
6	Etidocaine	11-4	30	150-212
7	Etidocaine	11-4	20	75-106
8	Etidocaine	11-4	20	300-425

due to the surface concentration of drug. After this initial burst, a constant delivery rate might be expected if the microcapsules were true wall and reservoir systems. An excellent correlation ($r = 0.989$) was obtained between the amount of surface etidocaine-HCl and the amount of drug released in one hour.

H. POROSIMETRY MEASUREMENTS

On similar projects, mercury porosimetry has been found to yield useful information on microcapsule morphology. A single test gives information on (1) bulk density, by a repeatable method on a small sample, (2) interparticle void volume, (3) pore volume, with volume associated with various restricted pore radii, and (4) skeletal density which defines closed pore volumes if the absolute density of the material is known. We sent samples of etidocaine-HCl microcapsules to Micromeritics Inc. for this analysis.

The data on the equivalent volumes for microcapsules of 70% etidocaine-HCl are shown in Table 22. For a bed of microcapsules of 106-300 micron sieve size, we assume that volumes with equivalent pore sizes of more than 10 microns are interparticle voids. Constrictions of less than 10 microns are considered to be pore volumes.

The bulk volume of microcapsules are dependent on the method of compaction. After loading the porosimeter tube with mercury at atmosphere pressure, volumes larger than 123 microns in diameter are filled by the porosimeter. The bulk volume of the microcapsules was estimated by carefully filling a small graduated cylinder with microcapsules. Extensive tapping was avoided. The bulk volume was 2.24 cm^3. Filling a mercury penetrometer gave a bulk volume of 1.98 cm^3/gm.

Physical property information is most useful as a quality control parameter. It is also useful as a correlating parameter with either <u>in vitro</u> drug release or a useful clinical property.

TABLE 22

POROSIMETRY DATA ON
Etidocaine-HCl Microcapsules
(70% drug, 106-300 μm)

	Equivalent Volumes (cm^3/gm)	
Bulk Volume (cylinder)	2.24	
Bulk Volume (porosimeter)	1.983	
Skeletal Volume	0.994	
Absolute Volume	0.833	(assume absolute d=1.2 g/cm^3)
Intrusion Volume	0.989	
Interparticle (>10 μm)	0.726	
Pores (total <10 μm)	0.263	
1.0 - 10 μm	0.024	
0.1 - 1.0 μm	0.074	
<0.1 μm	0.165	
*Closed Pore Volume	0.161	

* Difference of Skeletal and Absolute Volumes

I. STABILITY OF STORED MICROCAPSULES

The stability of the microcapsules under military field conditions had been questioned. Therefore preliminary experiments were performed to determine the stability of the drug (by *in vitro* assay) and the stability of drug release rate (also *in vitro*) as a function of various storage conditions. Relatively dry conditions of storage are required in order to maintain the drug within the microcapsules. Solid particles of the anesthetic hydrochlorides are known to be very stable. The base forms may be less stable. The poly-L(-)lactide is expected to be stable, but is hydrolyzed slowly in the presence of moisture. This is the basis of the biodegradation of the polymer to lactic acid in the body. As shown by the slow release of levonorgestrel from similar microcapsules (Contract No. N01-HD-0-2847) this *in vitro* and *in vivo* degradation requires several months to achieve a significant breakdown of a poly-L(-)lactide (R.S.V. approximately 1 dl/g) microcapsule wall.

Six storage conditions were chosen (Table 23) which covered a range of temperatures, with and without laboratory levels of humidity and light. Two separate containers of each drug were prepared. One container was to be opened at the end of the first contract year for assay and release testing. The other container held twice as much material and was to be used for two later analyses. American Can Company graciously supplied sufficient (retortable pouches M-1173 61-0.93004 heat-sealable bags) for dessiccant storage. These pouches consisted of Bartuf/Poly/Foil/L.D.P.E. Ambient light and moisture was achieved in glass vials capped with glass wool. Absence of light was achieved with black tape around the vials. Temperatures were 40°C (chemical oven), ambient (laboratory), 4°C (refrigerator). In the analysis of the data it must be remembered that different people, using slightly different techniques took the data in different years. Hence only gross changes may be significant. A condensed form of the lidocaine storage data is shown in Table 24. There is a stability of both lidocaine-HCl and lidocaine (base) as shown by the assay values. However there is an indication of faster release after storage at higher temperatures. The lidocaine hydrochloride microcapsules have a more constant rate of release than the lidocaine (base) microcapsules at high temperatures.

Etidocaine-HCl microcapsules are stable for two years, both in terms of the drug assay and the rate of drug release (Table 25). The bupivacaine-HCl microcapsule storage results are anomalous (Table 26). The drug assay values which are measured in methylene chloride appear unchanged. Also the relative quantity of drug released as a function of time is relatively

TABLE 23

SAMPLE STORAGE CONDITIONS

CONDITION	TEMPERATURE	HUMIDITY	LIGHT
1	40°C	Ambient	None
2	40°C	Desiccate	None
3	Ambient	Ambient	None
4	Ambient	Ambient	Ambient
5	4°C	Ambient	None
6	4°C	Desiccate	None

TABLE 24

STABILITY OF STORED LIDOCAINE MICROCAPSULES
(Percent of Assay Value Released at Specific Times)

Drug	Sample No.	Years Stored	Hours of Release	Original Data	Storage Conditions: 40°C Dark Unsealed	40°C Dark Sealed	R.T. Light Unsealed	R.T. Dark Unsealed	4 °C Dark Unsealed	4 °C Dark Sealed
Lidocaine ·HCl	06-1-50 150-212	1	1	36	60		47	41		
			6	81	82		90	78		
			24	83	83		98	83		
			Assay	47	50		43	42		
Lidocaine ·HCl	06-1-50 150-212	2	1	36	71	65	49	53	44	48
			6	81	92	93	91	93	90	91
			24	83	96	96	98	103	97	97
			Assay	47	45	43	43	41	43	43
Lidocaine base	11-1-30 212-300	1	1	16	92	82	29	27		
			6	45	95	103	73	73		
			24	79	95	104	98	98		
			Assay	72	71	68	65	66		

TABLE 25

STABILITY OF STORED ETIDOCAINE MICROCAPSULES
(Percent of Assay Value Released at Specific Times)

Drug	Sample No.	Years Stored	Hours of Release	Original Data	Storage Conditions: 40°C Dark Unsealed	40°C Dark Sealed	R.T. Light Unsealed	R.T. Dark Unsealed	4° C Dark Unsealed	4° C Dark Sealed
Etidocaine	06-2-50	1	1	4	1		3	4		
·HCl	150-212		6	13	14		13	16		
			24	24	37		32	34		
			Assay	(50)	46		49	50		
Etidocaine	06-2-50	2	1	4	3		2	4		
·HCl	150-212		6	13	19		43	34		
			24	24	93		83	98		
			Assay	(50)	64		65	64		
Etidocaine	11-2-30	1	1	22	18		14	25		
·HCl	106-300		6	58	63		49	68		
			24	99	93		83	98		
			Assay	66	64		65	64		

TABLE 26

STABILITY OF STORED BUPIVACAINE MICROCAPSULES
(Percent of Assay Value Released at Specific Times)

Drug	Sample No.	Years Stored	Hours of Release	Original Data	Storage Conditions: 40° C Dark Unsealed	40° C Dark Sealed	R.T. Light Unsealed	R.T. Dark Unsealed	4 °C Dark Unsealed	4° C Dark Sealed
Bupivacaine HCl	06-3-20 150-212	1	1	27	36		40	36		
			6	69	88		88	91		
			24	85	92		91	97		
			Assay	(80)	79		82	79		
Bupivacaine HCl	06-3-20 150-212	2	1	27	62	76	59	49	50	42
			6	69	109	114	103	103	108	97
			24	85	116	129	114	113	123	123
			Assay	(80)	72	67	82	72	67	67
Bupivacaine HCl	06-3-20	1	1	28	23		20	20		
			6	66	50		47	50		
			24	80	76		78	78		
			Assay	65	62		57	57		

constant. However, after two years, there is an increase in the total absorbance of the drug when measured in an aqueous diffusion system. This was observed in two separate sets of drug release experiments.

IV. IN VIVO STUDIES

A. INTRODUCTION

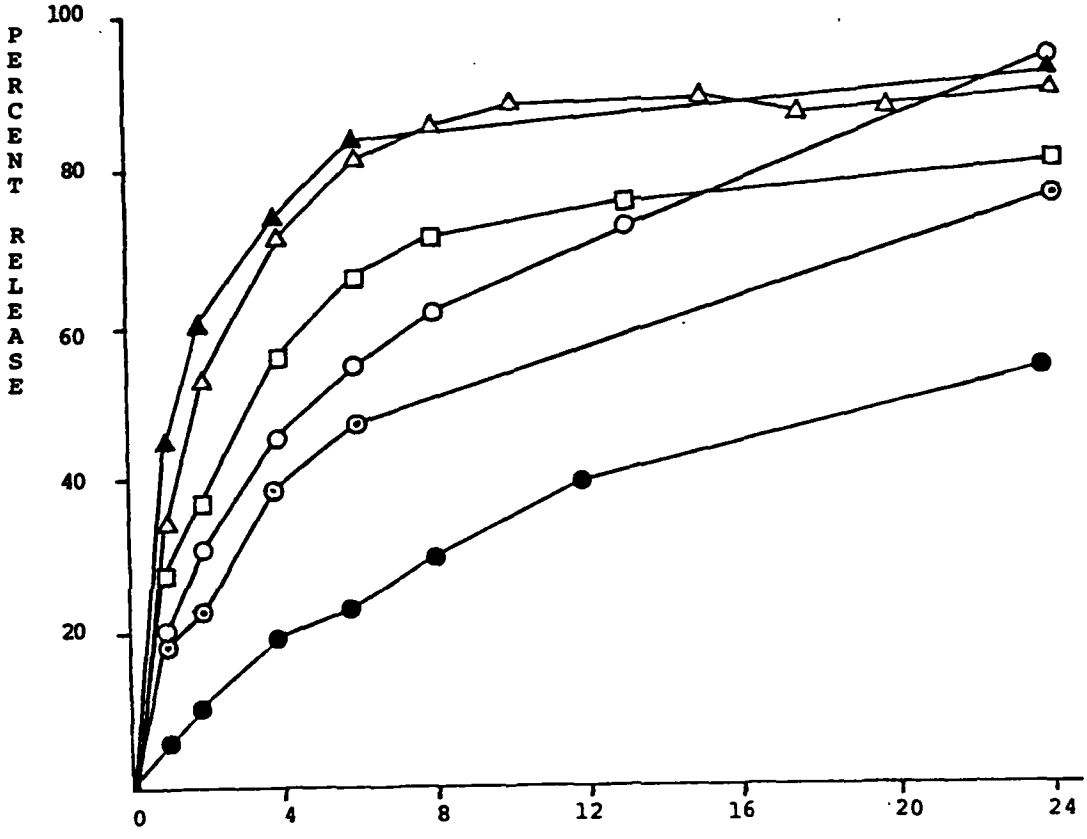
The in vitro studies clearly show that microencapsulated local anesthetic agents can be prepared and provide a sustained release of drug over a period of many hours. The objective of the in vivo studies was to demonstrate in animal models that the in vitro sustained release characteristic would provide a long duration of action, at least 24 hours, with no toxicity. Initial studies compared the acute toxicity of microencapsulated preparations with solutions of the pure drug and demonstrated a very significant (4 to 7 fold) reduction in toxicity of the microencapsulated drug. Since toxicity is related to the concentration of local anesthetic agents in the systemic circulation, an objective was to determine circulating levels concurrently with demonstration of duration of action. This could not be realized. Rabbits proved to be poor models for testing local anesthesia, and rats and guinea pigs which are good models for local anesthesia are not convenient for pharmacokinetic studies. Therefore the blood studies were done in rabbits and the duration studies were carried out using the guinea pig intradermal wheal and subcutaneous injection models, and the classical rat sciatic nerve model.

In summary, these studies clearly demonstrated that the sustained release of drug from microcapsules minimizes systemic toxicity by preventing the initial peak in circulating concentrations of drug and prolongs the duration of action to the point that 48 hours of local anesthesia can be established in the rat sciatic nerve model without inducing convulsions. This is a dramatic improvement over solutions of the pure drug.

B. SELECTION OF MICROCAPSULES FOR IN VIVO STUDIES

Microcapsules of lidocaine, etidocaine, and bupivacaine hydrochlorides were used in preliminary in vivo experiments in Contract DAMD17-80-C-0110. However, insufficient quantities of these microcapsules were available for continued in vivo experiments. Based on the in vitro diffusion of various sizes of microcapsules and the need for a range of release rates, the material shown in Figure 26 was chosen for in vivo studies. The calculated quantity of available microcapsules is also given in this figure.

Figure 26 Drug Release and Quantities Available for *In Vivo* Studies



Drug	Run	Drug %	Size (μm)	Bed %	Grams
▲ Lidocaine-Base	11-1-30	75	150-212	8	10
△ Lidocaine-HCl	06-1-50	77	150-212	35	70
□ Bupivacaine-HCl	11-3-30	64	150-250	50	23
○ Etidocaine-HCl	11-2-30	66	106-300	30	58
⊙ Etidocaine-HCl	06-2-30	(70)	74-106	28	3
● Etidocaine-HCl	11-4-30	47	150-212	19	36

C. TOXICITY TESTING

1. Systemic Toxicity

Since the systemic toxic effects of local anesthetic agents are acute reactions (convulsions and death due to respiratory failure) the toxicity may be assumed to be related to the peak concentration of anesthetic in the circulating system. Microencapsulation, which provides sustained release, should decrease this peak value. However, in order to have a longer-acting anesthetic it is also anticipated that more drug may have to be used in the encapsulated form. For acute toxicity testing, intraperitoneal injection of the solution and microcapsules was used since a relatively large cavity was required for injection of the microcapsules. This route of administration bypasses the slow absorption of a subcutaneous injection. Oral and intravenous modes are clearly inappropriate.

Toxicity data for lidocaine, bupivacaine, and etidocaine was requested from NIOSH-TIC (Occupational Safety and Health Technical Information System). Over 500 abstracts were received. A recent article by deJong and Bonin (1981) yielded excellent data on the convulsive and lethal doses of all three compounds when injected intraperitoneally in mice. The data are:

Drug	n	CD50 (mg/kg)	LD50 (mg/kg)
Lidocaine	48	111.0 ±6.3	133.1 ±3.3
Bupivacaine	41	57.7 ±2.7	58.7 ±2.0
Etidocaine	60	54.9 ±2.2	64.4 ±3.0

John Bonin at Tufts University, Boston, Mass. was contacted about the experimental details. Our major difference is the requirement to use a larger needle in order to inject a suspension of microcapsules. Also a suspending vehicle is required to maintain a uniform suspension of microcapsules. We found the suspending vehicle used by NCI to be appropriate. However this 0.3% hydroxypropylcellulose solution in saline is no longer produced by Carter-Glogan Laboratories. It was produced under contract to NCI and six bottles of this material were supplied to us, gratis, by Nathaniel Greenberg at NCI. When this supply was exhausted we prepared our own vehicle using hydroxypropylcellulose obtained from Hercules, Inc., Wilmington, Del., (Klucel HF, Lot 3291). This material contained a small quantity of silica.

a. Preliminary LD50/CD50 Experiment

Following the method of deJong and Bonin (1981) we obtained 10 outbred virgin female white Charles River CD-1 mice. They were ordered as 8-week mice and used at 10 weeks. However, the

weights were 24.8 ±1.9 gm instead of the 30 gm mice used by deJong and Bonin. As expected, an 18-gauge needle is less than ideal for intraperitoneal injections. However, it is necessary in order to inject a suspension of microcapsules. By tipping the head back after making the injection, while keeping the needle in the cavity, the solution does not leak out as the needle is removed. Finger pressure is also applied over the puncture hole as the needle is removed.

Injection of 0.30 ml of 11.2 mg/ml lidocaine-HCl solution (3.36 mg drug) in hydroxypropylcellulose (HPC, 0.3%) and saline (0.9%) caused convulsions about 4 minutes after the injection. Very shallow breathing followed but the mouse was back on its feet within 20 minutes. This is approximately the LD50 as published by deJong and Bonin.

A suspension of microcapsules was injected into the last three mice. This suspension was successfully injected within 5 minutes of adding the suspending vehicle. However, the suspension was not uniform. The suspension (2 ml) contained 50 mg/ml of 50% drug microcapsules of 150-212 microns. This is a 5% suspension containing 25 mg lidocaine-HCl per milliliter. Injection of 0.42 ml (10.5 mg drug, if uniform suspension) did not cause convulsion or death in the first two mice. The remaining material was rather difficult to suspend and 0.30 ml caused the animal discomfort.

In the laboratory the remaining drug was measured in the syringes and vial used for the microcapsule suspension. The results confirmed the non-uniformity of the suspension.

In order to improve the uniformity of the delivery of microcapsules the vial was changed to a 1 dram bottle which would accept a GC septum cap. This gave fewer available crevices for storage of powder and gave a better view of the suspension. The vial was prepared with 97.6 mg of the same microcapsules, and 2.0 ml of suspending vehicle was added. Five aliquots of 0.3 ml were removed, dried, and weighed. Each aliquot should contain 18.2 mg (14.6 mg of microcapsules and 3.6 mg of NaCl and hydroxypropylcellulose). The weighed containers gave weight increases of 18.9 ±2.5 mg.

Based on these experiments a protocol was written for the injection of 64 mice with four levels of microcapsules and four levels of soluble lidocaine-HCl (8 mice/group with 4 trials at increasing doses and 4 at decreasing doses). The method was readily modified for other drugs, since lidocaine concentrations are higher than those required for the other drugs.

AD-A166 796 LOCAL ANESTHETIC MICROENCAPSULATION(U) BIOTEK INC 2/2
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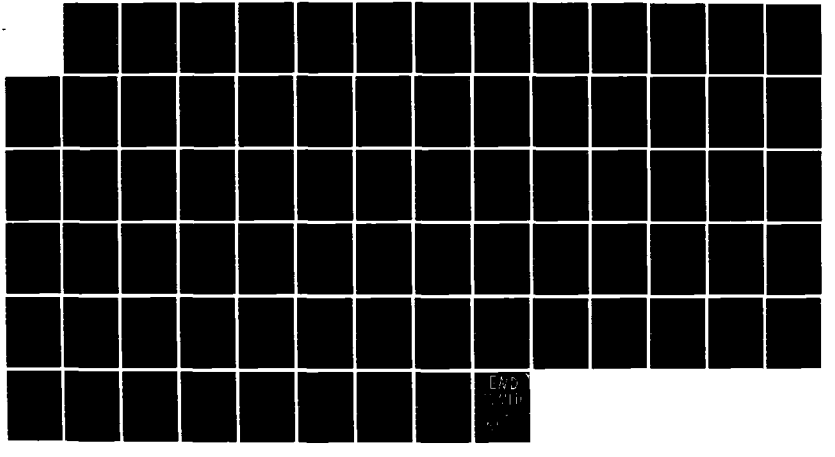
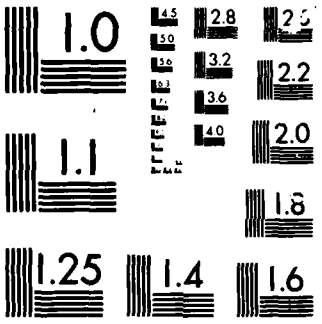




MICROCOPY CHART

b. Lidocaine-HCl Microcapsule Toxicity

One hundred CD-1 female mice were ordered at 56 days old. Their weights were 26.0 ±1.4 gm (n=20), and therefore no adjustment was made for the differences in weights. Doses were prepared at 30% incremental differences around the CD50 to LD50 doses. The experiment was performed when the mice were 59 days old. From the data of the series of 64 mice, a lower concentration of solution and higher suspension loading was required. All experiments were performed in one day. Solution and suspension concentrations were 112 mg/11 ml and 127 mg/1.7 ml, respectively. Volumes were 0.23, 0.30, 0.39, 0.50 ml. The suspension was used within 10 minutes of the time that the aqueous vehicle was added. Three cages were used below each dose holding cage, to hold injected (normal), convulsed, and dead mice. Mice were also marked according to the dosing group. The lower solution dose was accomplished with 0.18 ml of the standard solution. More concentrated suspensions (12%) were prepared to allow an injection of 0.5 ml to contain the increased dose. The data of this experiment is shown in Table 27.

Each syringe and bottle was labelled and the solids content were combined, dried, and weighed after the experiment. The calculated quantity remaining was 43.2 mg. This weight included the calculated quantity of HPC and NaCl. The weighed quantities were 47.1 ±6.4 mg. Thus, we conclude that most of the microcapsules were uniformly suspended and delivered.

The important factor is the difference in toxicity between the pure and encapsulated drug, when injected intraperitoneally. Based on both convulsive and lethal toxicity end points, the encapsulated drug is approximately five times less toxic than the pure drug. The lower toxicity was anticipated and more drug is expected to be required to produce anesthesia for a prolonged period of time.

c. Etidocaine and Bupivacaine Microcapsule Toxicity

Etidocaine and bupivacaine-HCl microcapsules were compared to the soluble anesthetic using the same general protocol.

For etidocaine-HCl, solutions were prepared at 40 mg/10 ml, and suspensions were prepared at 40 mg/2.0 ml of vehicle. These were dispensed in 0.23, 0.30, 0.39, 0.50, 0.50, 0.39, 0.30 and 0.23 ml volumes. For etidocaine-HCl microcapsules, a higher dose was required (80 mg and 3.1 ml vehicle, for 0.50 ml dosing). The animal weights for this experiment were 26.4 ±1.2 grams (57 days old). The data are shown in Table 28.

For bupivacaine-HCl microcapsules, the mice were 64 days old and weighed 28.9 ±1.2 grams. Bupivacaine-HCl solutions

TABLE 27

TOXICITY OF LIDOCAINE·HCl IN SOLUTION AND MICROCAPSULES

	Solution [mg (drug)/kg (mouse)]					Suspension* [mg (drug)/kg (mouse)]				
	68	89	112	150	195	402	523	671	872	1132
Not convulsant	7	5	0	0	0	8	2	0	0	0
Convulsant	1	3	8	7	3	0	6	6	4	1
Dead	0	0	0	1	5	0	2	2	4	7

CD50**	91.6±6.7	488±42
LD50**	181±12	838±79
LD50/CD50	1.99	1.72

M.C. Safety Factor	
$CD_{mc}/CD_{soln.}$	5.33
$LD_{mc}/LD_{soln.}$	4.60

* Microcapsules of 150-212 micron, 50% lidocaine·HCl, Run 6-1-50

** Calculated by method of Berkson, 1953, following Example 2

TABLE 28

TOXICITY OF ETIDOCAINE·HCl IN SOLUTION AND MICROCAPSULES

	Solution (mg/kg)				Suspension* (mg/kg)				
	34.5	44.8	58.3	75.8	114	148	192	250	325
Not Convulsant	7	4	2	0	7	7	3	2	0
Convulsant	1	3	4	0	1	1	3	4	0
Dead	0	1	2	8	0	0	2	2	8

CD50	47.3±4.5	191±21
LD50	62.3±6.9	260±34
LD50/CD50	1.32	1.36
M.C. Safety Factor		
$CD50_{mc}/CD50_{soln}$	4.04	
$LD50_{mc}/LD50_{soln}$	4.17	

* Microcapsules of 106-300 microns, 70% etidocaine·HCl, Run 11-2-30
Drug Assay of 66%

were prepared at 40 mg/10 ml of vehicle. Suspensions were prepared at 100 mg of microcapsules in 2.6 ml of vehicle. The high dose was achieved in separate studies using more concentrated solutions and suspensions. The data are presented in Table 29.

As was observed with lidocaine, microencapsulation of etidocaine and bupivacaine significantly reduced systemic toxicity. For etidocaine microcapsules the CD50 increased 4.04 fold and the LD50 increased 4.17 fold compared to the solution. For bupivacaine both toxicity measures increased 6.7 fold. This may be related to the slower drug release of the bupivacaine microcapsules compared to the lidocaine and etidocaine preparations.

d. Comparison to Literature Values

The toxicity measures for solutions of local anesthetics determined in the present study are compared to those of deJong and Bonin (1981) in Table 30. In all cases the CD50 in the present study was slightly lower than in the earlier study. The LD50 for both lidocaine and bupivacaine were somewhat higher than those of deJong and Bonin (1981). Our temperature was maintained at 21°C rather than 38°C and our vehicle contained hydroxypropylcellulose and therefore had a higher viscosity than the saline used by deJong and Bonin.

2. Local Toxicity (CPK Analysis)

An increase in creatine phosphokinase (CPK) in blood is a sensitive indicator of muscle damage. This approach was used by Zener and Harrison (1974) to follow muscle damage after IM injection of a 10% lidocaine solution in human volunteers. CPK values increased about 7-fold from 40 to 300 IU/1. During the study in which 4% lidocaine was injected intramuscularly into rabbits (see Section III.D.3.b), additional blood samples were taken for normal and 6-hour CPK values. All values were high, compared to humans, with the norm being about 3000 IU/1. Six hours after injection of microcapsules, the CPK was 17,000 IU/1, whereas 6 hours after the injection of a solution the CPK was 32,500 IU/1.

These tests were performed by a local clinical laboratory, using a kinetic uv determination of enzyme activity. Creatine phosphokinase (CPK) analyses were then performed at BIOTEK using the Sigma CPK-UV diagnostic kit (No. 45). The CPK activity in serum was determined at 6 and 24 hours following the intramuscular injection of 3 ml of vehicle, 4% lidocaine-HCl solution or 4% microencapsulated lidocaine-HCl. The data are shown in Table 31.

TABLE 25

TOXICITY OF BUPIVACAINE·HCl IN SOLUTION AND MICROCAPSULES

	Solution (mg/kg)					Suspension* (mg/kg)				
	31.9	41.3	53.9	70.1	91.1	184	239	311	404	525
Not Convulsant	8	4	1	0	0	8	6	1	2	1
Convulsant	0	4	7	3	0	0	2	7	4	1
Dead	0	0	0	5	8	0	0	0	2	6

CD50	41.5±6.2	280±44
LD50	67.6±4.9	453±40
LD50/CD50	1.6	1.6
M.C. Safety Factor		
$CD50_{mc}/CD50_{soln}$	6.7	
$LD50_{mc}/LD50_{soln}$	6.7	

* Microcapsules of 150-250 micron of 70% bupivacaine·HCl, Run 11-3-30
Drug Assay of 60%

TABLE 30

COMPARISON OF PRESENT TOXICITY DATA
WITH deJONG AND BONIN (1981)

		Present Study	deJong/Bonin
Lidocaine·HCl	LD50	181±12	133.1±3.3
	CD50	91.6±6.7	111.0±6.3
Etidocaine·HCl	LD50	62.3±6.9	64.4±3.0
	CD50	47.3±4.5	54.9±2.2
Bupivacaine·HCl	LD50	67.6±4.9	58.7±2.0
	CD50	41.5±6.2	57.7±2.7

TABLE 31

Creatine Phosphokinase (CPK) Levels
After Lidocaine-HCl Injection in Rabbit
(3 ml, I. M.)

		Mean ± S.E.M. in I. U./liter		
	n	Before	6 hours	24 hours
Vehicle	6	527 ± 80	2606 ± 481	2228 ± 430
Microcapsules	6	377 ± 53	1466 ± 195	1868 ± 372
Solution	6	345 ± 56	2698 ± 246	4097 ± 542
Saline	3	610 ± 118	1260 ± 295	1335 ± 734

At 24 hours lidocaine Solution vs Vehicle, $t = -2.7$, $p<0.025$.

3ml of 4% lidocaine-HCl injected as a Solution or as a Suspension of Microcapsules of 47% Drug and 150-212 Micron Size.

The normal CPK values are much higher in rabbits (400 IU/liter) than in humans (normals 40 IU/liter). Following an intramuscular injection of hydroxypropylcellulose vehicle (0.3% in saline, using 0.5 ml at each of 6 locations), there was significant increase of CPK activity. The increase in CPK following treatment with microcapsules was very similar to vehicle treatment. However the lidocaine solution gave the highest CPK values ($p<0.025$). These data suggest that the microencapsulated lidocaine caused no more tissue damage than the vehicle alone and much less damage than the solution.

Data from subcutaneous administration of lidocaine solutions and microcapsules show a similar increase of CPK by lidocaine solution. However the CPK activities are significantly lower for this mode of administration. CPK values 24-hours after microcapsule injection are 410 IU/liter, and for lidocaine solution the value is 2,400 IU/liter.

Therefore, both systemic and local toxicity are reduced by microencapsulation of local anesthetic agents. These data support the hypothesis that the local anesthetic agent is released from microcapsules in a sustained manner resulting in lower peak blood and tissue concentrations.

D. CIRCULATING LEVELS OF LIDOCAINE IN RABBITS

1. Introduction

The results of the acute toxicity tests indicated that the sustained release of drug from the microcapsules observed *in vitro* also occurred *in vivo*. Thus encapsulation has presumably reduced the peak concentration achieved in the systemic circulation. An initial objective was to simultaneously test this hypothesis and determine the duration of local anesthesia in rabbits. As will be shown in Section III.E.1 and 2, the rabbit is not a suitable model for testing local anesthesia. In addition, guinea pigs and rats are not suitable for repeated blood sampling during anesthesia testing. Therefore, separate models were needed to investigate both hypotheses. In this section the methods for analyzing blood concentrations of local anesthetics and the animal procedures are described first. Then the results are presented.

In summary, the data clearly show that the peak concentrations are much lower following administration of microencapsulated drug than solution. This supports the conclusions of the acute toxicity tests and suggests that a significantly larger dose of drug may be delivered with microcapsules. Thus a longer duration of action should be achieved with microencapsulated drug, without inducing systemic toxicity.

2. Methods of Analysis

Four separate methods were used to measure the concentration of local anesthetic agents in rabbit blood: two gas chromatographic (GC) procedures using a flame ionization detector (FID), performed at BIOTEK, Inc; an enzyme immunoassay system obtained from Syva and used at BIOTEK; and a gas chromatographic method using a mass spectrometer (M.S.) detection system which was performed at USAIDR.

The first gas chromatographic system used at BIOTEK, Inc. was based on that of Mather and Tucker (1974). Local anesthetic agents were extracted from rabbit blood plasma using a solution of 10 ng lidocaine-HCl per ml of methylene chloride when etidocaine was measured and etidocaine-HCl as the internal standard when lidocaine was measured. The method requires an extraction into ether from an acidified sample, followed by an extraction into methylene chloride from an alkaline sample. Dry ice is used to freeze the extraction systems. Finally, a G.C. syringe needle must go through the aqueous layer to reach the conical methylene chloride layer in a Reacti-Vial to pick up the final sample.

An aliquot of methylene chloride extract was injected into a Perkin Elmer 990 gas chromatograph equipped with a 6 foot glass column packed with 10% OV-17 on 80/100 Supelcoport (obtained from Supelco, Inc., Bellefonte, PA). The carrier gas was helium at a 25 ml/minute flow rate and column temperature was 210°C. The output of the FID detector was recorded and analyzed on a Hewlett-Packard 3390-A integrator. A sample record is shown in Figure 27.

A background sample of blood was used to generate a standard curve for the extraction procedure with varying amounts of lidocaine in the presence of an internal standard of etidocaine. From the graph, Figure 28, it can be seen that both peak areas and peak heights show excellent linearity versus the amount of lidocaine added to the 1 ml blood sample. Therefore, the peak height could be used to calculate the amount of circulating lidocaine.

On both graphs the X-intercept is 0.149 micrograms for peak area and 0.154 micrograms for peak height. This sets a theoretical lower limit of lidocaine detection at about 0.15 ug/ml.

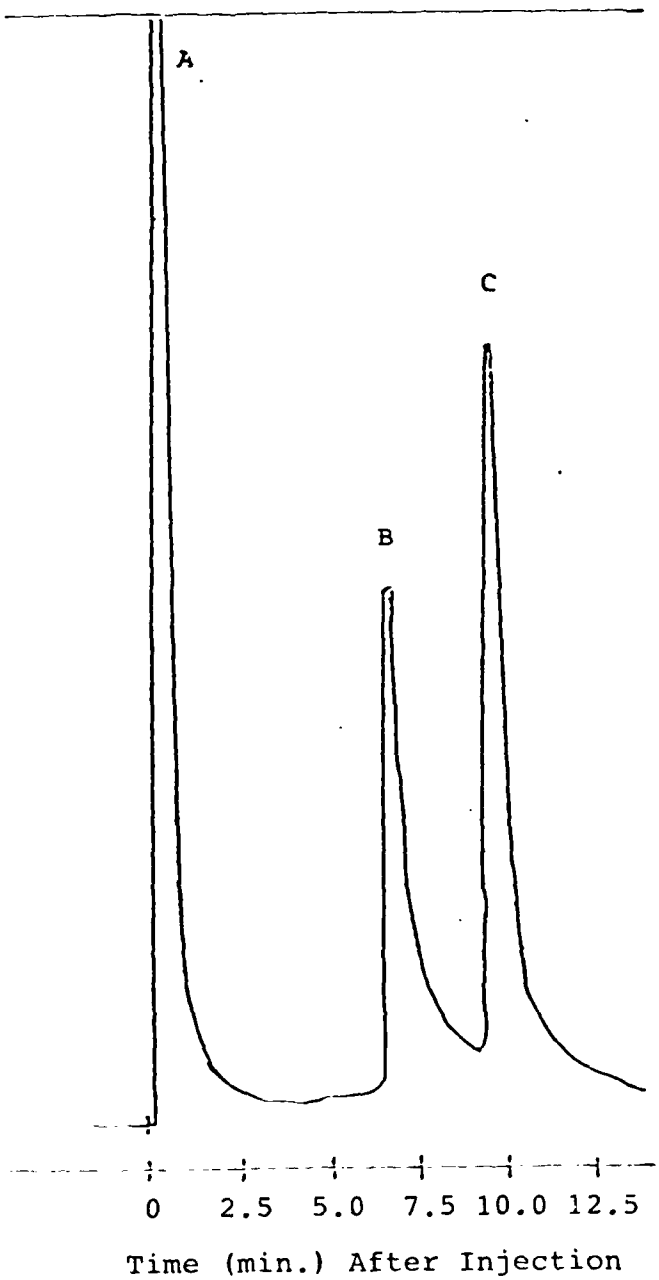
The GC/MS analysis of amide anesthetics and their metabolites was performed on a limited number of samples at USAIDR and the results were not immediately available to us. Similarly, the original GC/FID procedure is time-consuming. Fortunately, circulating levels of lidocaine are measureable using an enzyme immunoassay procedure which was developed by Syva for cardiac patient monitoring (EMIT). This is a rapid, stat, procedure. We therefore used this procedure on one set of rabbits to generate preliminary data to indicate a release of lidocaine from a set of lidocaine-HCl microcapsules.

We modified the method for use with the equipment in our laboratory. The standard method uses a pre-dilution (6-fold, 50 ul to 300 ul) of the serum. For the later time samples we omitted this dilution. The method is simple, sensitive, and specific. However, it would not measure etidocaine or any metabolites of the lidocaine.

The method of Mather and Tucker (1974) for GC analysis of amide anesthetics has been used by several investigators. However, the method is complex. In discussions with other researchers (Wynkoop, USAIDR and Moore, Harvard Dental School), the results are variable. Recently published modifications include Park, et al (1980) who evaporate the methylene chloride of the final extraction and redissolve in methanol. Also Holt, et al (1979) describe a simple procedure in which the original plasma is made basic and lidocaine is extracted into chloroform.

FIGURE 27

DETECTION AND SEPARATION OF LIDOCAINE/ETIDOCAINE
ON 10% OV-17 COLUMN @ 210°C
(1.0 µl of solution containing 2 µg of each drug)



Peak	Assignment
A	Solvent - CH_2Cl_2
B	Lidocaine - 2 µg
C	Etidocaine - 2 µg

Format	y = mx + b y = L/E at E= 1.25µg x = L (µg/ml)
□ Peak Height	m = 0.9086 b = -0.14 r = 0.9996 x (y=o) = 0.154
○ Peak Area	m = 0.6739 b = -0.1005 r = 0.99989 x (y=0) = 0.149

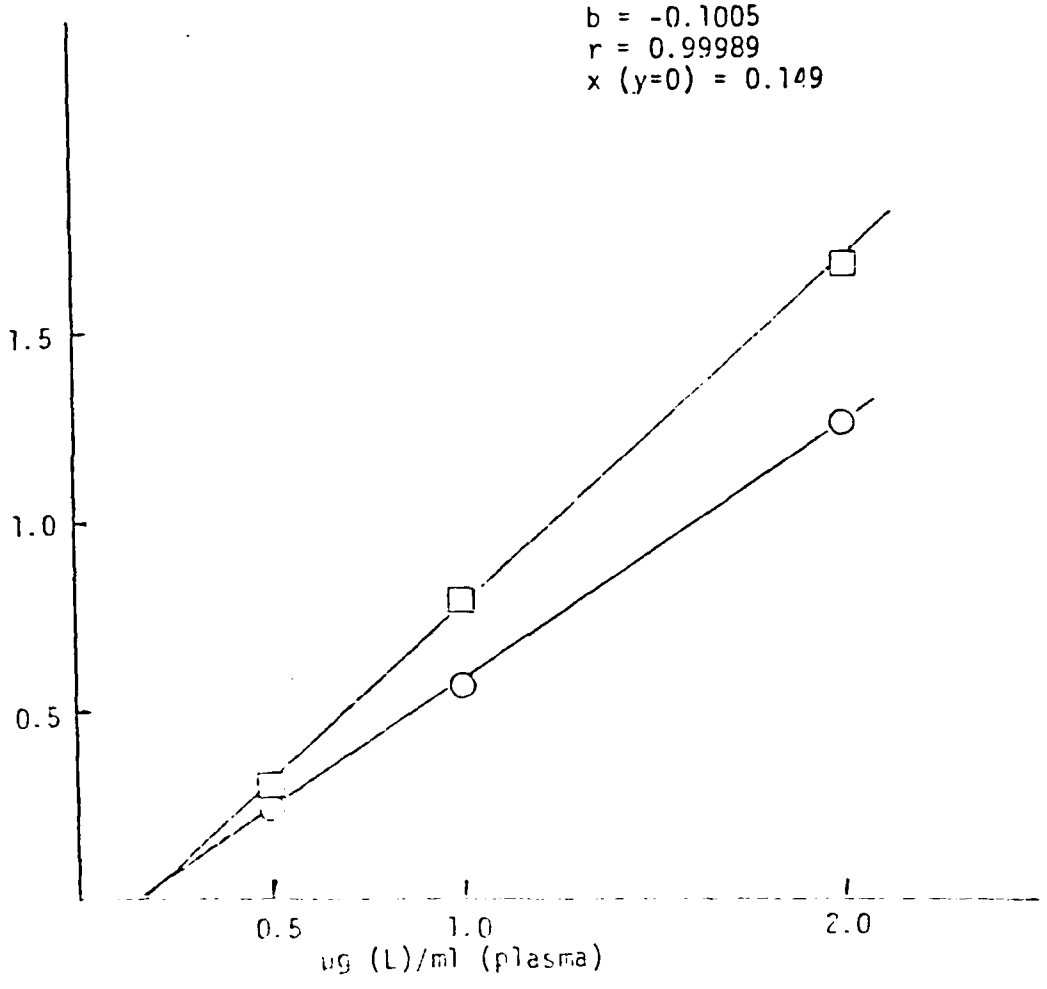


Figure 28 Standard Curve for Lidocaine (L) in Plasma with Etidocaine (E) as Internal Standard

Use of other detectors, such as mass spectrometry undoubtedly increase the method sensitivity (Park, et al 1980), and may allow use of a more simplified extraction procedure. Also, metabolites may be measured by more sophisticated instrumentation. For these reasons we continued to send blood samples to Dr. Wynkoop at USAIDR for GC/MS analysis.

In order to simplify the GC/FID procedure we tested the method described by Holt, et al (1979). The advantage of this method is the single step extraction. A 200 ul aliquot of plasma or serum is added to 200 ul of 2M Tris base and mixed. The lidocaine is extracted into 50 ul of chloroform containing 2.14 ug/ml of etidocaine base as an internal standard by vortexing for 60 seconds. The organic phase is separated by centrifugation at 4070 xg, 0°C, for 60 minutes. A 2 ul aliquot of the chloroform layer is injected into a 1/4 inch glass column, 6 feet long, packed with 3% OV 101 on 80/100 Supelcoport (Supelco, Inc., Bellefonte, PA). The column is pretreated as described by Holt, et al (1979). Chromatography conditions for the Perkin-Elmer 990 were: oven temperature, 210°C; manifold and injector temperatures, 250°C; and helium carrier gas flow, 35 ml/minute.

This method also could be used to measure the level of toxic metabolites (e.g. MEGX). A standard curve and typical chromatograms of a standard and a serum extract in water and serum are shown in Figure 29.

3. Animal Procedures

a. Anesthetic Implantation in Rabbits

In early studies the simultaneous evaluation of local anesthesia in an avulsive wound and determination of blood levels of lidocaine was attempted. The general method used is summarized below and a detailed description follows.

During the development of the in vivo procedures, it was found that ketamine (Ketaset™) could not be used as the general anesthetic since it gave two peaks on the gas chromatograph, one of which had the same retention time as lidocaine. Furthermore, since the general anesthetic was present in much larger concentration than lidocaine, the lidocaine peak was totally masked. Ether is an acceptable general anesthetic, but is difficult to work with. Therefore, thiamylal sodium (Biotal™, a short acting barbituate) was tried. This was found to provide good short-acting anesthesia and showed no conflicting peaks in a chromatogram of extracted plasma which was taken one hour after thiamylal injection.

Adult New Zealand white rabbits were placed under a short-acting general anesthetic. A 1 cm incision was made with

Figure 29

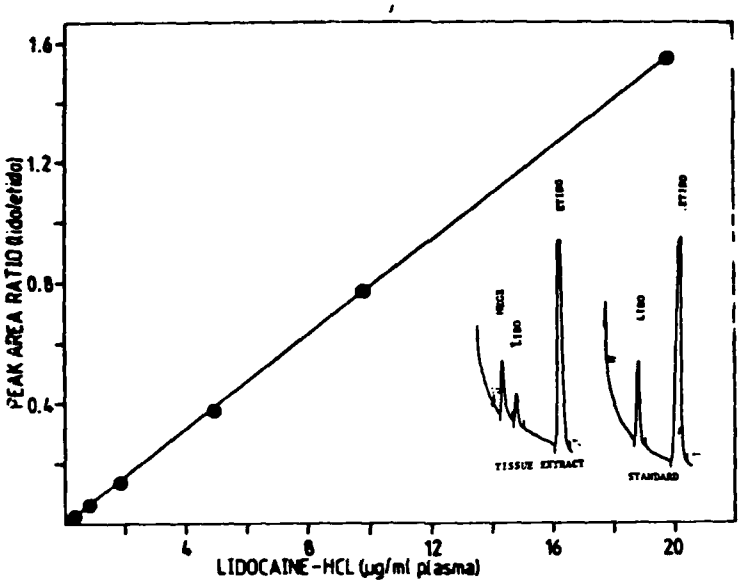


Figure 29. Lidocaine-HCl standard curve
(gas chromatography)
MEGX, monoethylglycinexylidide;
LIDO, lidocaine;
ETIDO, etidocaine internal standard

surgical scissors. The sub-dermal membranes (including fascia) were cut to expose a standard pocket (elliptical of about 0.5 cm and 0.4 cm, major and minor radial dimensions). Dry powder (drug microcapsules or neat crystals) was then poured into this cavity while the skin was held away (and up from the muscle) with forceps. The wound was then closed with a single suture. Concentric circles were marked from this suture. At various times after drug placement, tactile response was measured as a twitch at various points along these circles. No twitch or shiver indicated anesthesia. Blood was withdrawn from the ear vein at various times after drug implantation and the circulating level of the drug was measured following the method of Mather and Tucker (1974).

The following procedure was used to conduct the test.

	Task	Approximate Duration (seconds)
a.	Inject Bio-tal	30
b.	Wash site with Betadine and Zepharin	30
c.	Make incision	90
d.	Place test material in wound (time = 0)	15
e.	Close wound	30
f.	Mark circles	45
		Total Time = 4 minutes

Details of the procedure are given below.

Instruments were sterilized and the thigh area of the rabbit was shaved using small animal clippers with a No. 40 blade. One blood sample was drawn to determine any residual drug or interfering materials before the local anesthetic was implanted.

Blood was taken from the marginal ear vein, after dilation of the vessel with 50% xylene and 50% methanol. The blood was drawn into a 20-ml syringe containing anti-coagulant (3 drops of 14% K EDTA), using an EZ-Set with a 23-gauge needle. With sufficient care and practice, the rabbit can be bled the required six times in one day. The blood is then transferred to a serum separator tube, refrigerated within one hour, and centrifuged within one day. Plasma samples were then kept refrigerated or frozen until analyzed.

Bio-tal™ was injected intravenously into the ear which was not to be used for bleeding for several hours. Two to three milliliters of 2% w/v of Bio-tal™ (thiamylal, sodium, M.I. 9035) was injected, depending on the weight of the rabbit (3.5 to 5.5 kg, i.e. approximately 11 mg/kg). This solution was injected over a period of 30 seconds, and the animals were

unconscious for about 5 minutes. This allowed enough time to disinfect the test site, make the incision, place the test material in the incision, close the wound and draw circles on the surrounding tissue. Within 15 minutes after injection, the animal is usually fully conscious and standing normally on his hocks. At this time, and thereafter, the rabbit has normal reflexive action to tactile stimulus (i.e., at areas distant from the locally anesthetized wound).

For the incision, the skin was lifted with forceps and cut to a length of 1 cm with surgical scissors. This incision does not extend to the fascia, and additional membranes are picked up with forceps and cut away until the muscle mass is reached. The forceps were then rotated to develop a pocket which is at least 0.6 cm in diameter. A packet of drug crystals of microcapsules is then poured carefully into the pocket. Finally the wound is closed with a single 4-0 gut suture. Any drug on the skin surface is wiped away with a moist gauze. Bloods are drawn at 1/2, 1, 2, 4, 6, and 24 hours after the drug application.

b. Anesthetic Injection in Rabbits

Following discussions with Dr. Judson Wynkoop and Robert Miller, a method of hypodermic syringe injection of lidocaine was developed which was similar to that used by the USAIDR personnel. Thus, their previous data on blood levels of lidocaine in rabbits following intramuscular injection of various quantities of lidocaine-HCl solution could be used as control data. After reviewing the in vitro release data of various lidocaine microcapsules, we decided to perform a pilot study with three rabbits injected with lidocaine solution and three rabbits with lidocaine (base) microcapsules.

Six injection sites were chosen and marked on the thigh (2 rows of 3 sites, 1 cm between rows and 0.75 cm between sites in a row). The syringe was marked at 1.2 cm and inserted to this depth. Three milliliters were injected as 0.5 ml injections. The suspension was prepared as 213 mg of 75% lidocaine (base) microcapsules (11-1-30, 150-175 microns) in 4 ml of the hydroxypropylcellulose vehicle used for the systemic toxicity (LD50) studies. This was designed to give 3 ml of a 4% lidocaine (base) injection in free and microencapsulated forms. The solution was prepared as 198 mg of lidocaine-HCl which was dissolved in 4 ml of HPC vehicle.

The blood samples were drawn at standard times (10 and 30 minutes, and 1, 2, 4, 6, 10, 24, and 48 hours. The analysis of lidocaine was measured by a GC/MS procedure at USAIDR.

The intramuscular injection of lidocaine as solution and microcapsules proceeded smoothly. The force required for the

IM injection sometimes led to some sieving effects, with a packed bed of microcapsules left in the syringe. When this happened we resuspended the microcapsules in additional vehicle and re-injected. In the first experiment with these microcapsules, we weighed the solids remaining in the syringe and vial after injection of 3 ml of the suspension. We found 102 mg and calculated that 97 mg should have been left. Therefore, we believe that the method does deliver the appropriate amount of microcapsules.

Since a negligible quantity of lidocaine was measured in the systemic circulation following the injection of lidocaine (base) microcapsules, in the next study a lidocaine-HCl microencapsulated preparation was used. Based on our earlier measurement of blood levels of lidocaine from similar lidocaine-HCl microcapsules by the Mather and Tucker method, we anticipated measurable values of lidocaine from the microencapsulated product in this new experiment.

The suspension was prepared as 340 mg of 45% lidocaine-HCl microcapsules (06-1-50, 150-212 microns) in 4 ml of HPC vehicle. This was designed to give 3 ml of a 4% lidocaine-HCl injection in free and microencapsulated form. The solution was prepared as 160 mg of lidocaine-HCl which was dissolved in 4 ml of HPC vehicle.

The injections of lidocaine-HCl solutions and microcapsule suspensions were performed as in the previous experiment. The samples were sent with dry ice by courier to USAIDR for analysis.

At this point future experiments were discussed with Dr. Wynkoop at USAIDR and we decided to increase the number of rabbits in this study from three to six. At this point the effect of subcutaneous injections of the same solution and microcapsule suspension was also evaluated. These samples were stored frozen at BIOTEK for several months and finally analyzed at BIOTEK by the Holt, et al (1979) procedure.

4. Circulating Anesthetic Results

a. Microcapsule Implantation Study

Lidocaine-HCl microcapsules (06-1-50, 150-212 um, 20 mg as lidocaine-HCl) or lidocaine-HCl drug crystals (20 mg) were implanted in a surgical wound as described above. Blood samples were drawn at specific intervals and the plasma samples were analyzed by the method of Mather and Tucker (1974), also as described above.

The results are shown in Figure 30. Mature New Zealand white rabbits of about 4 kg were used, and the results were normalized to a 4 kg rabbit. The implanted microcapsules deliver lidocaine to the system more slowly than the implanted lidocaine crystals. The peak value for microcapsules is about 50% of the peak level using pure anesthetic crystals. In these experiments, the peak lidocaine crystal blood level was 450 ng/ml. This is about 10% of the toxicity limit set for intravenous lidocaine infusion when used for suppression of ventricular arrhythmias. However, the true peak may not occur at 30 minutes. After two hours, the blood level from the microcapsules tends to be higher than for the crystals.

A similar experiment was performed with etidocaine-HCl microcapsules (06-2-30, 74-106um) and crystals. This data is shown in Figure 31. Blood values for the three experiments with etidocaine microcapsules are remarkably constant from 1/2 to 6 hours. Etidocaine crystals show a surge of drug reaching the systemic circulation within 30 minutes. By the 4 and 6 hour times, the blood levels are equivalent for crystal and microcapsule implantation. The blood values (ug/ml) are normalized for a 4 kilogram rabbit.

b. Microcapsule Injection Studies

In the first microcapsule injection experiment, 120 mg of lidocaine (base) was injected as microcapsules (11-1-30, 150-212 um) in an hydroxypropylcellulose vehicle, and as a solution of the hydrochloride in the same vehicle. The samples were sent to USAIDR for GC/MS analysis of circulatory lidocaine and lidocaine metabolic products. Unfortunately, the metabolites of lidocaine are at lower levels in the rabbit and could not be quantified in this experiment.

The results of circulating lidocaine are shown in Table 32. By omitting the initial GC/FID data at USAIDR the averages are shown in Table 32. Some early blood values from injections of lidocaine solutions were in the toxic range (> 5 ug/ml) as defined from use of lidocaine to suppress ventricular arrhythmias. However, no toxic effects were noted in these rabbits. The fast decrease of blood lidocaine values is consistent with the 95 minute half life of this drug. The drop from 4.38 to 0.52 ug/ml in one-half hour in Rabbit 367 is not explainable. Fortunately, lidocaine is very stable in stored serum (Levine, Blanke, Valentour, 1983). The most remarkable result was the small quantity of circulating lidocaine when lidocaine base microcapsules were injected.

FIGURE 30 LIDOCAINE PLASMA CONCENTRATIONS AFTER IMPLANTATION OF DRUG CRYSTALS AND MICROCAPSULES

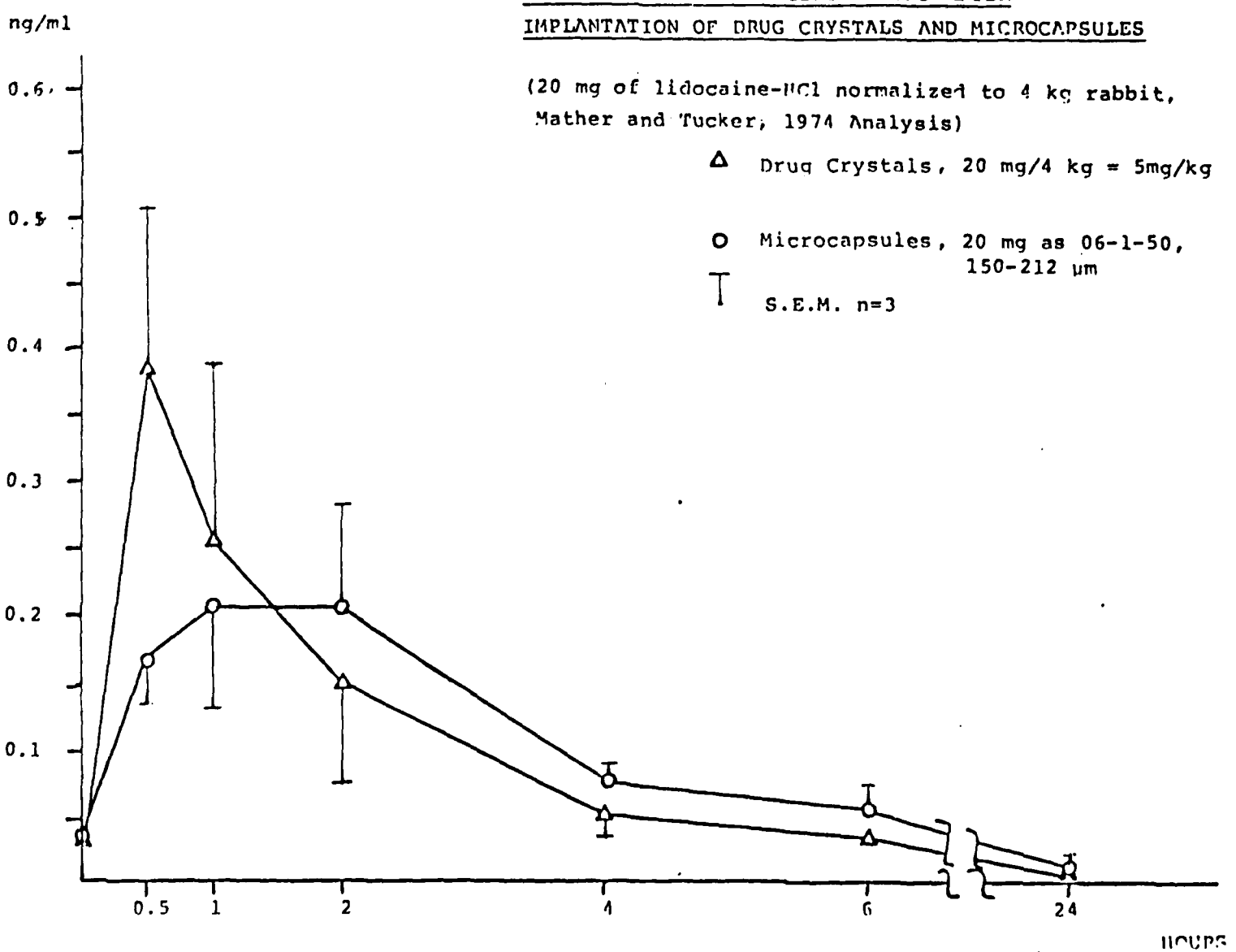


FIGURE 31 ETIDOCAINE PLASMA CONCENTRATIONS AFTER IMPLANTATION OF CRYSTALS AND MICROCAPSULES

(20 mg of Etidocaine-HCl normalized to 4 kg Rabbit, Mather and Tucker, 1974 Analysis)

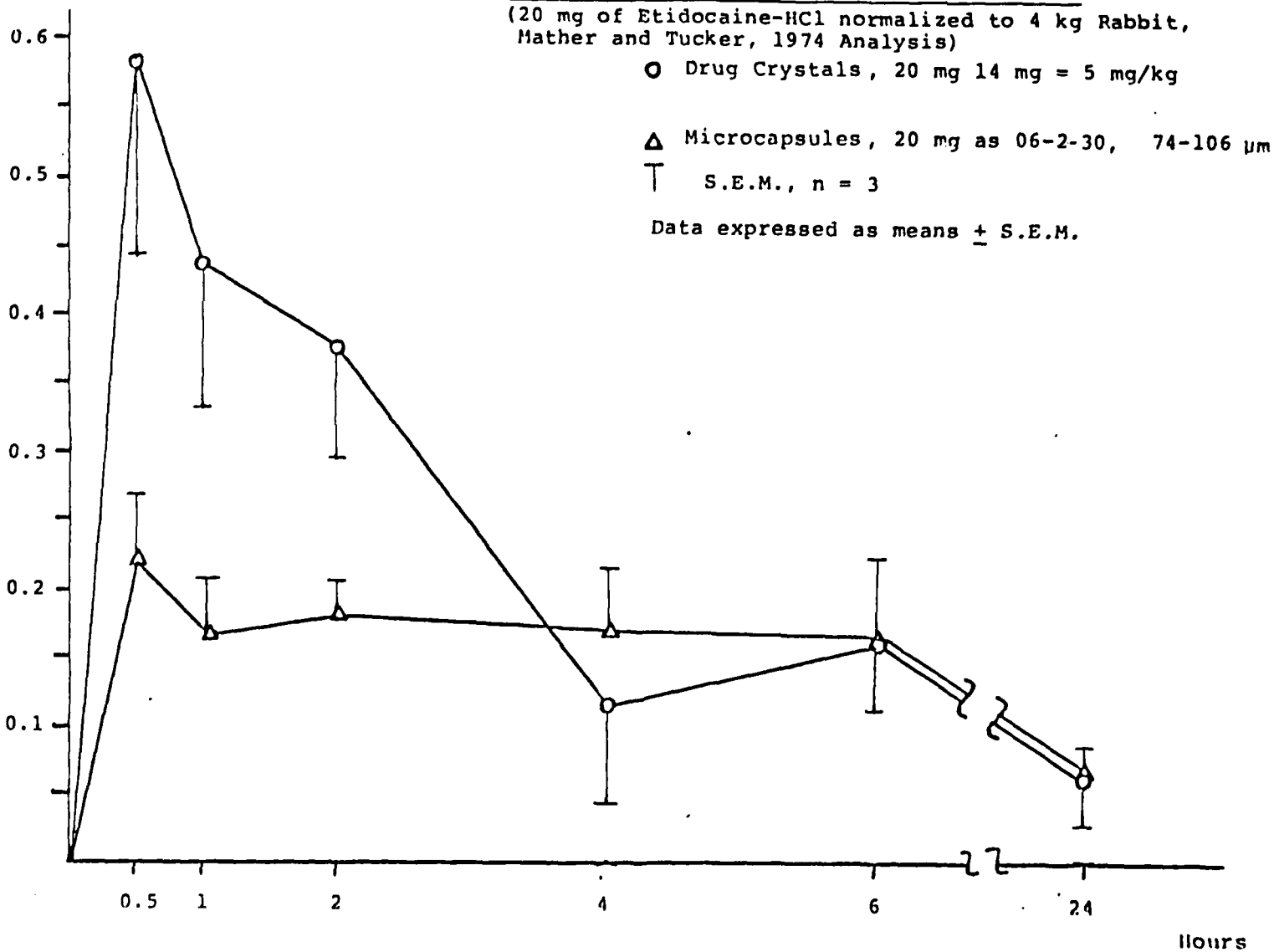


TABLE 32

CIRCULATING LEVELS OF LIDOCAINE FOLLOWING IM INJECTION OF SOLUTIONS AND MICROCAPSULES OF LIDOCAINE BASE

(GC/MS AT USAIDR)

Rabbit No.	Weight (kg)		Exp. Date	Serum Levels (µg/ml) at 0:10	0:30	1:00	2:00	4:00	6:00	10:00	24:00	48:00
LIDOCAINE SOLUTION INJECTION												
365	4.9		6/10	4.72	5.58	2.33	1.22	0.72	0.27	0.05	0.04	--
365	4.9		6/14	5.90	6.97	2.94	1.52	0.90	0.34	0.05	0.04	--
367	4.4		6/7	3.85	4.38	0.52	0.30	0.09	0.25	0.13	0.02	--
		→	Mean	4.82	5.64	1.93	1.01	0.57	0.29	0.08	0.03	
			SEM	0.59	0.75	0.72	0.37	0.24	0.03	0.03	0.01	
365(FID)			6/10	1.69	1.21	0.73	0.42	0.20	0.04	0.09	0.05	
LIDOCAINE MICROCAPSULE INJECTION *												
364	4.2		6/10	0.26	0.18	0.10	0.09	0.07	--	0.06	0.01	--
367	4.4		6/14	0.26	0.26	0.20	0.19	0.10	0.05	0.09	0.05	0.01
368	4.4		6/7	0.30	0.05	0.05	0.04	0.04	0.03	0.01	0.00	0.00
		→	Mean	0.21	0.16	0.12	0.11	0.07	0.04	0.05	0.02	0.01
			SEM	0.08	0.06	0.04	0.05	0.02	--	0.02	0.02	--

* Lidocaine (base) microcapsules, 11-1-30, 75% drug, 150-212 microns, 120 mg of lidocaine (base)

Since lidocaine is primarily metabolized by the liver, the area under the curve (AUC) was expected to be similar for any administration of the same quantity of drug. Thus, the encapsulated base showed very little systemic bioavailability in one day. Furthermore, the small amount that was measured as circulating lidocaine was rapidly released to the circulation.

In the next intramuscular injection experiment, lidocaine-HCl microcapsules were injected which had an in vitro release rate of 50% in two hours (06-1-50, 150-212 um). Based on our earlier measurement of blood levels of lidocaine from these microcapsules, we anticipated measurable values of lidocaine from this experiment. A dose of 120 mg in a mature New Zealand rabbit was again used.

The only toxic reaction occurred with Rabbit 350. About 7 minutes after the injection of the lidocaine solution, the rabbit went into a tonic extension convulsion. She was removed from the restrainer and appeared quite cyanotic for several minutes (7-10 minutes). She had an arched back and neck rigidity for about 20 minutes after the injection. The rabbit was bled, outside the restrainer at about 15 minutes. She appeared to be out of danger at that time, although still convulsing.

An enzyme immunoassay was set up to verify a measurable blood level with microcapsules before proceeding with other animals. The result of this assay is shown in Figure 32, and it confirmed our previous results with implanted microcapsules and the GC/FID analysis.

The results of the GC/MS procedure of USAIDR are shown in Table 33 and Figure 33. For calculation of the mean and for plotting, the one hour data point of Rabbit 167 on 9/20/82 was removed. It did not seem reasonable, with respect to the other GC/MS values, nor with respect to our EMIT values. We also interchanged the ten minute data on 9/13/82 for Rabbits 350 and 190. Rabbit 350 convulsed violently and almost died. The sample was taken about five minutes late, and probably went into the wrong tube.

The final set of samples were analyzed at Biotek by the method of Holt. This was a second set (3) of intramuscular injections and a set (3) of similar subcutaneous injections.

In general, the progression of drug levels as a function of time was regular for each animal. However there was a large variation between animals. A summary of the data is shown in Table 34 and 35, Figures 34-35. As expected, the blood levels were initially lower for injections of the microencapsulated

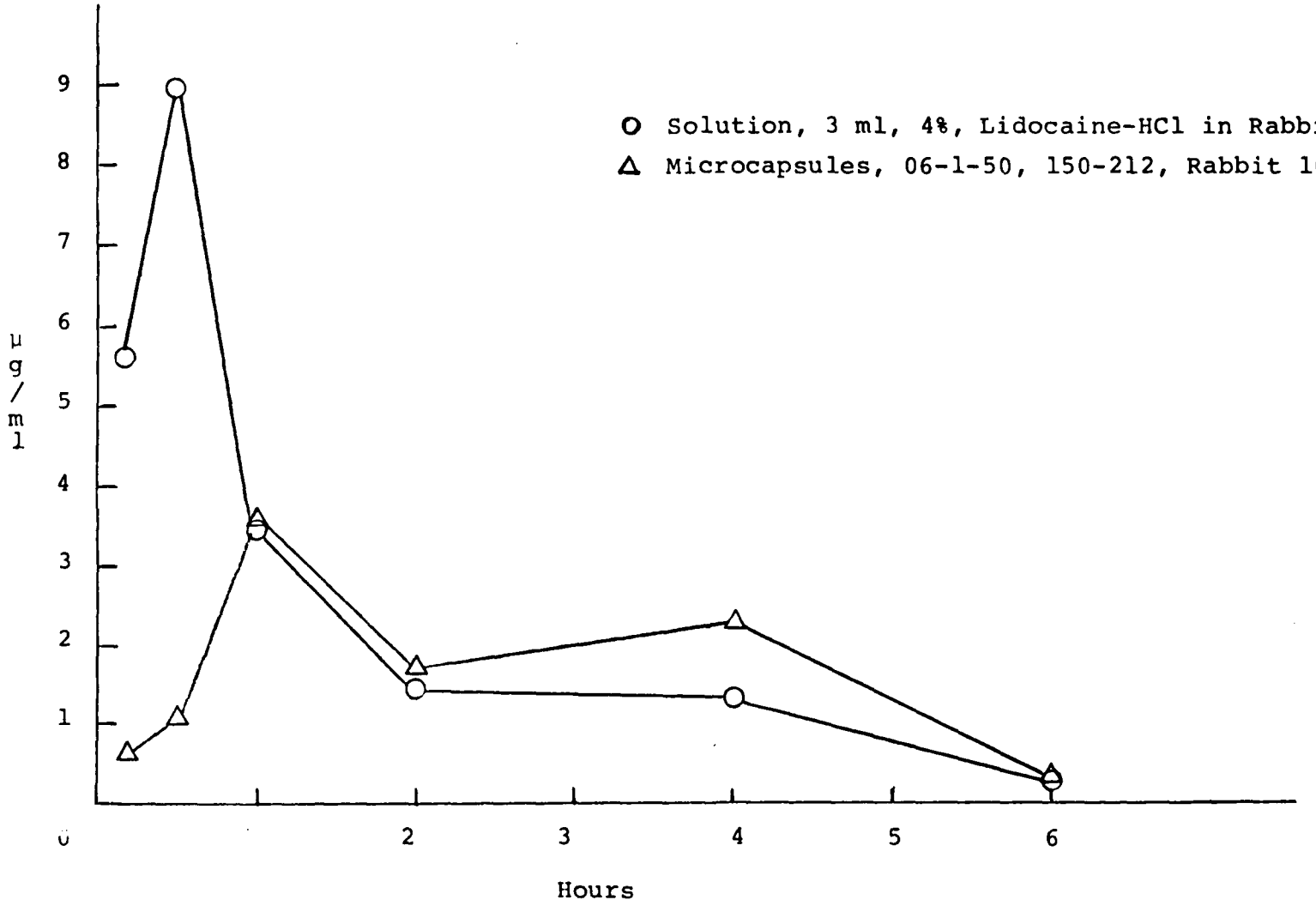


Figure 32 Serum Levels of Lidocaine after I.M. Injection of 120 mg of Drug as Solution or Microcapsules (EMIT Assay at BIOTEK)

TABLE 33

CIRCULATING LEVELS OF LIDOCAINE FOLLOWING IM INJECTION OF SOLUTIONS AND MICROCAPSULES OF LIDOCAINE-HCl (GC/MS AT USAIDR)

Rabbit No.	Weight (kg)	Exp. Date	Serum Levels (µg/ml) at 0:10	0:30	1:00	2:00	4:00	6:00
LIDOCAINE SOLUTION INJECTION								
167	5.2	8/31	3.83	2.16	1.09	0.78	0.22	0.03
350	4.4	9/13	8.71	1.91	1.34	0.50	0.25	0.16
186	5.3	9/20	4.19	2.88	0.59	0.25	0.17	0.06
Mean		→	5.57	2.31	1.01	0.51	0.21	0.08
SEM			±1.57	0.29	0.22	0.15	0.02	0.04
186	(EMIT)	9/20	5.6	9.0	3.4	1.4	1.3	0.3
LIDOCAINE-HCl MICROCAPSULE INJECTION*								
169	5.4	8/31	0.75	0.80	0.91	0.78	0.17	0.03
190	4.5	9/13	0.16	1.02	0.89	0.67	0.28	0.11
167	5.2	9/20	0.28	0.41	(0.15)	0.61	0.22	0.09
Mean		→	0.40	0.74	(0.90)	0.69	0.22	0.08
SEM			±0.18	0.18	----	0.05	0.03	0.02
167	(EMIT)	9/20	0.6	1.1	3.6	1.7	2.3	0.3

* Lidocaine-HCl microcapsules 06-1-50, 47% drug, 150-212 µm

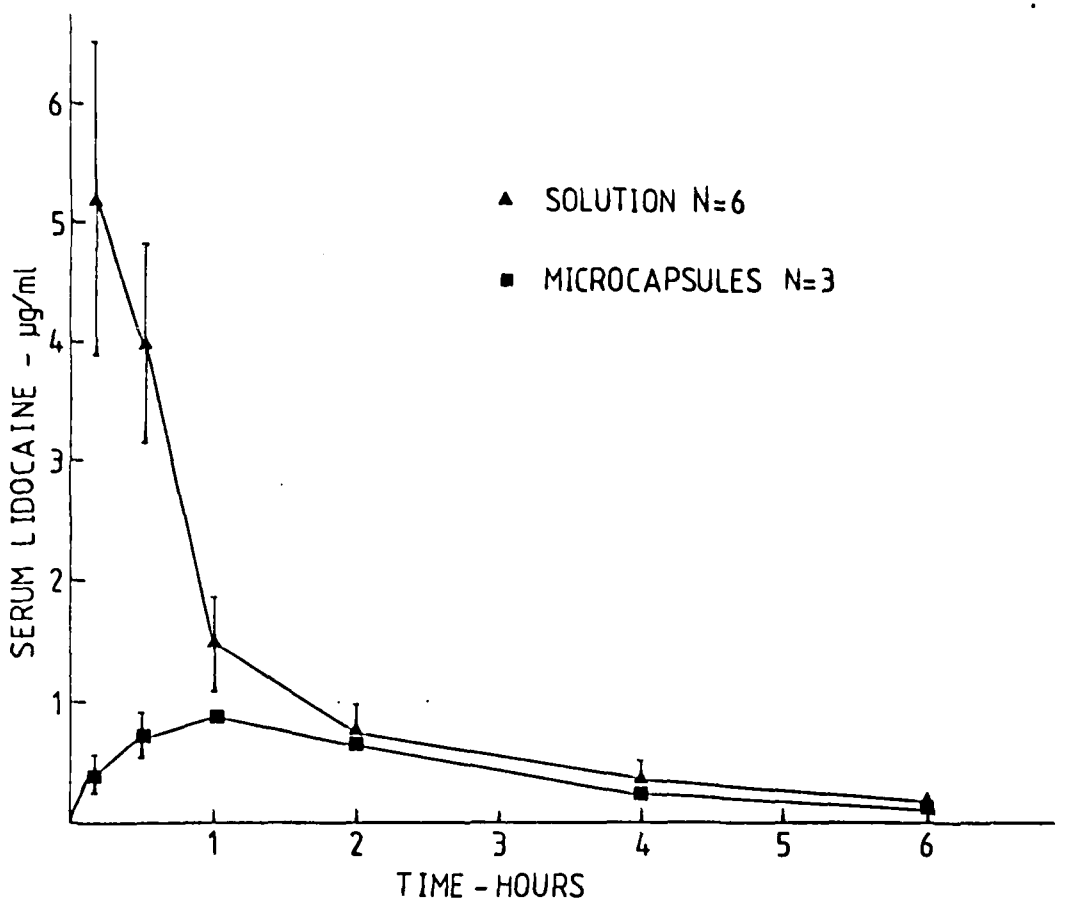


Figure 33 Serum Levels of Lidocaine After Injection of 120 mg of Lidocaine-HCl as Solution or Microcapsule Suspension (3 ml of 4% Lidocaine-HCl as Either a Solution or as a Suspension of Microcapsules of 47% Drug and 150-212 µm Were Injected I.M. in Rabbits) GC/MS Assay at USAIDR. Vertical lines are standard error of the mean.

TABLE 34

CIRCULATING LEVELS OF LIDOCAINE FOLLOWING IM INJECTION OF SOLUTIONS AND MICROCAPSULES OF LIDOCAINE-HCl

(GC/FID at Biotek, Holt, et al 1979)

Rabbit No.	Weight (kg)	Exp. Date	Serum Levels (μg/ml) at 0:10	0:30	1:00	2:00	4:00	6:00	10:00
LIDOCAINE SOLUTION INJECTION									
294	4.7	11/8	14.09	7.70	3.45	2.54	1.63	0.60	0.16
275	4.7	11/10	2.58	8.98	3.58	2.26	1.14	0.48	0.54
293	4.2	11/18	8.06	7.60	5.62	3.15	1.02	0.73	0.12
Mean			8.24	8.09	4.22	2.65	1.26	0.60	0.27
S.E.M.			3.33	0.44	0.70	0.27	0.18	0.08	0.13
LIDOCAINE-HCl MICROCAPSULE INJECTION									
350	4.5	11/8	0.77	1.90	3.34	2.85	1.33	0.72	0.15
295	4.5	11/10	0.45	0.70	0.98	1.00	0.63	0.25	0.17
296	4.2	11/18	0.84	2.06	2.52	2.68	1.37	0.83	0.20
Mean			0.69	1.55	2.28	2.18	1.11	0.60	0.17
S.E.M.			0.12	0.43	0.69	0.59	0.24	0.18	0.02

* Lidocaine-HCl microcapsules 06-1-50, 47% drug, 150-212 μm

TABLE 35

CIRCULATING LEVELS OF LIDOCAINE FOLLOWING SC INJECTION OF SOLUTIONS AND MICROCAPSULES OF LIDOCAINE-HCl

(GC/FID at Biotek, Holt, et al 1979)

Rabbit No.	Weight (kg)	Exp. Date	Serum Levels (µg/ml) at 0:10	0:30	1:00	2:00	4:00	6:00	10:00
LIDOCAINE SOLUTION INJECTION									
350	4.5	11/22	0.97	1.26	1.45	1.31	0.68	0.31	0.12
275	4.7	11/8	8.30	8.15	7.67	4.08	2.05	1.38	0.23
278	3.8	12/13	3.93	3.57	5.79	2.91	1.38	6.91	0.05
Mean			4.40	4.33	4.97	2.77	1.37	0.87	0.13
S.E.M.			2.13	2.02	1.84	0.80	0.40	0.31	0.05
LIDOCAINE-HCl MICROCAPSULE INJECTION*									
294	4.7	11/22	3.17	2.04	4.21	1.55	0.99	0.44	0.18
295	4.5	12/8	1.38	1.59	1.46	0.78	0.43	0.11	0.00
293	4.2	12/13	0.21	0.68	0.71	0.78	0.24	0.02	0.04
Mean			1.59	1.44	2.13	1.04	0.55	0.19	0.07
S.E.M.			0.86	0.40	1.06	0.25	0.23	0.13	0.05

* Lidocaine-HCl microcapsules 06-1-50, 47% drug, 150-212 µm

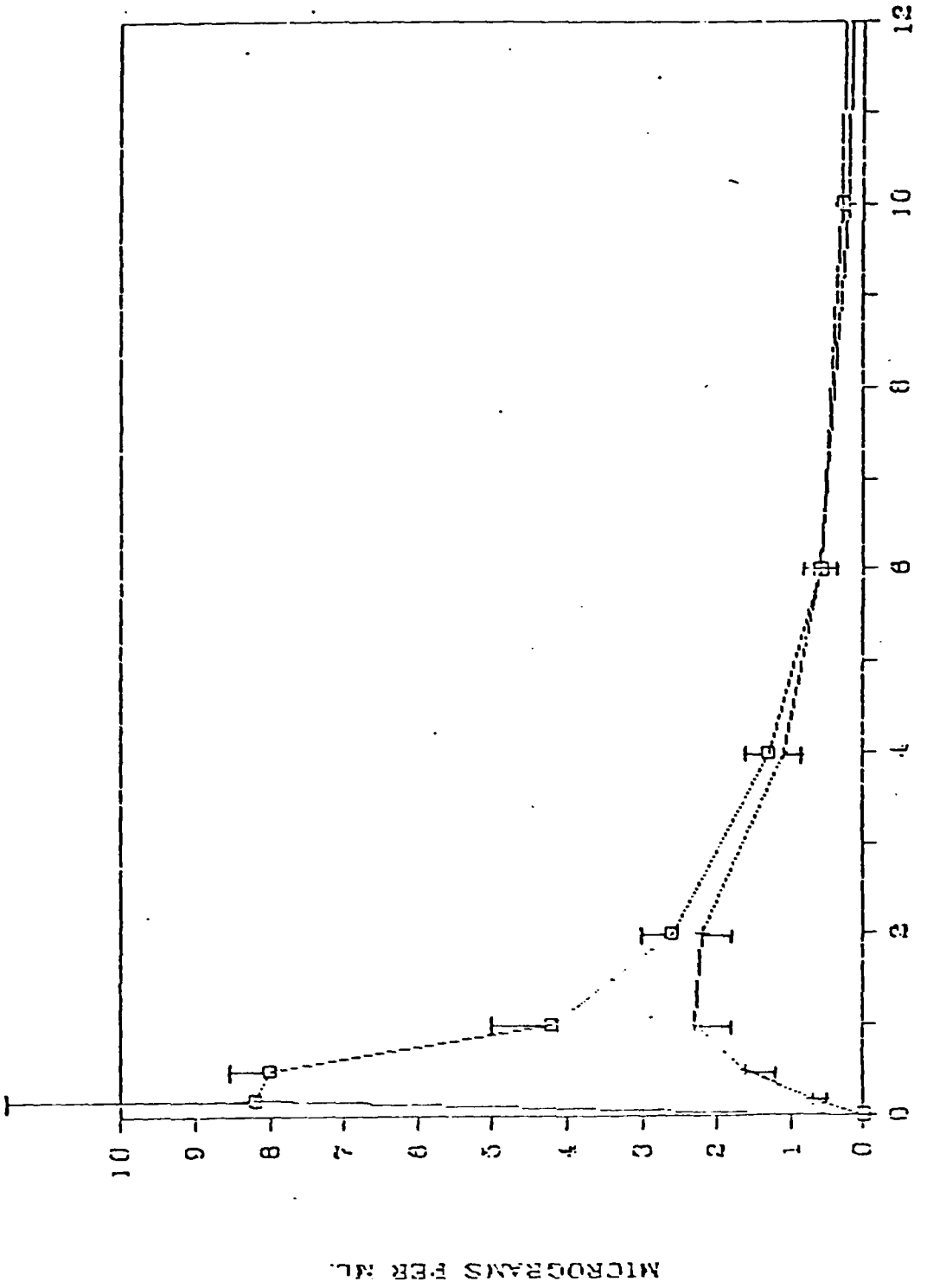


Figure 34 Lidocaine-HCl Serum Levels Following Intramuscular Injection of Solution or Microcapsule Suspension (mean ±S.E.M.)

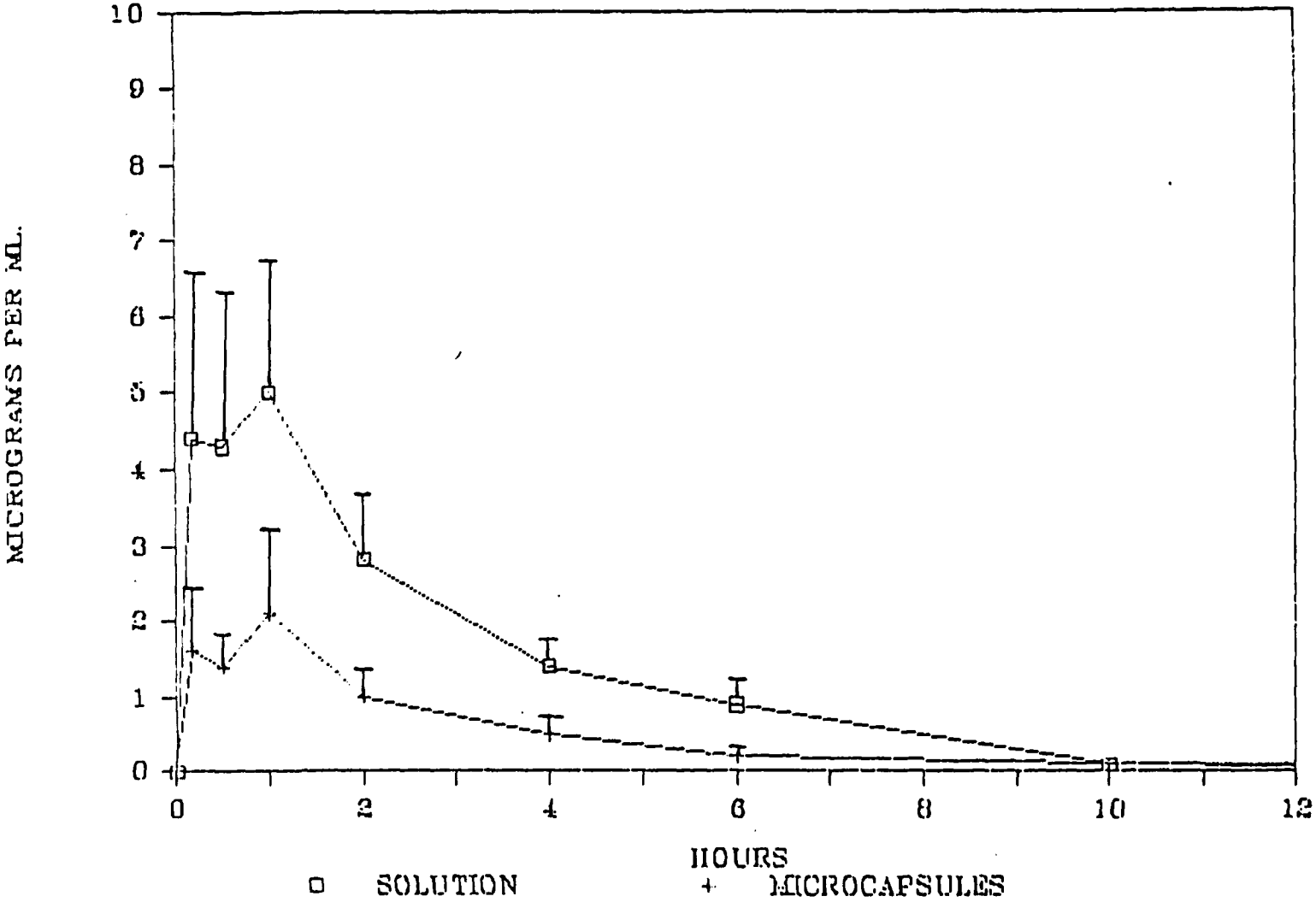


Figure 35 Lidocaine-HCl Serum Levels Following Subcutaneous Injection of Solution or Microcapsule Suspension (mean ±S.E.M.)

drug. However, the later blood levels for the microencapsulated drug injections did not exceed the values for the solution injections. The results also show that the subcutaneous injections were more variable than those for the intramuscular injection. Also, the absorption was slower from the S.C. than from the I.M. site.

Again, the areas under the curves (AUC) were unequal for equal quantities of drug injected. However, in this local anesthetic application, the overwhelming of tissue storage with the subsequent spilling over of unbound anesthetic into the circulation is unwanted. There is a significant advantage to maintaining the anesthetic bound to this local tissue (deJong, 1977, p. 220). However, the lack of equivalency of areas under the curve (AUC) is worrisome. Lidocaine is primarily metabolized in the liver and local tissue metabolism is believed to be minimal. However, the AUC for lidocaine-HCl microcapsules appears to be less than for the same quantity of drug in solution. Also, the lidocaine (base) microcapsules generated almost no bioavailable drug by the analysis of the sera collected for one day.

From all of this data, the trend is clear. The potential systemic toxicity of a rapid peak level of lidocaine is eliminated by microcapsulating the lidocaine-HCl. The anesthetic is released from the microcapsules over a period of several hours. The data are too sparse and variable to warrant a mathematical analysis, such as described by Smolen, et al, (1979), or Bjornsson and Desjerdins (USAMRDC contractor, see Shand, et al, 1981).

E. MEASUREMENTS OF ANESTHESIA

Because of difficulties with reproducibility of the avulsive wound model and tactile determination of local anesthesia, an E.M.G. method for determining local anesthesia was developed. The contract was modified to include this new method.

Dr. Judson Wynkoop at USAIDR also had developed a tactile stimulator which is based on a spring-loaded syringe needle. This device was kindly sent to us for reproduction. Appropriate engineering sketches were drawn, and the device was fabricated by a local machine shop. A 15-gauge needle was cut and rebevelled, such that it protruded 5 mm from the end plate.

Rabbits were chosen initially because blood levels of the local anesthetic agent were to be determined during potency and duration testing. Needle electrodes were placed subcutaneously 2 cm apart along the midline of the back, near the sacral-lumbar junction. The direct and integrated electromyogram (EMG) was recorded form these electrodes using a Narco Biosystems Physiograph with a high gain preamplifier and an envelope type, full-wave rectifying integrator. Respiration was recorded from two needle electrodes placed subcutaneously on opposite lateral chest walls using the Narco impedence pneumograph. Local anesthetic agents were administered either subcutaneously or intradermally on the upper thigh and lower back.

1. Intradermal Rabbit Studies (Solutions)

For intradermal tests, four anesthetic solution doses and a vehicle control were tested simultaneously in separate wheals. The tactile stimulus was a needle prick delivered by the USAIDR device. When the observer delivered the stimulus, the event pen of the recorder was activated. If a twitch response was observed, the remote switch for the event marker was immediately released. If no response occurred, the event marker was not released for at least 3 seconds. Thus,the observed twitch response, EMG, and respiration were simultaneously recorded. Later, the event marker was automatically tripped by the travel of the barrel of the USAIDR stimulator. It was also found necessary to build a rabbit restrainer which allowed access to the hind quarter of the rabbit, but minimized random movement of the rabbit.

Each test trial consisted of five stimuli to each injection site and five trials were performed over a 30-minute period. Data were recorded as either number of negative responses (local anesthesia) or percentage of the 25 possible responses that were negative. If a stimulus resulted in an immediate, integrated EMG response of 2 mm or more, as determined by vertical relationship to the event marker, it was defined as positive. The height of the response in mm was also measured.

The recordings show that the integrated EMG has a stable baseline, and response to the stimulus is easily detected. This is superior to the direct EMG response. The respiratory response to stimulation is a short gasp which is the most sensitive measure. However, since the baseline recording is very unstable, this response was not used.

There was a major problem with this method. Five rabbits were tested on two sessions at least 7 days apart, with one trial on each side during each session. Following injection of 0.2 ml of saline vehicle intradermally, 17.2% ±4.6 of the observed twitch responses were negative. When lidocaine-HCl was being tested the control sites gave 27.8% ±2.3 negatives, and when procaine-HCl was being tested 7.6% ±4.4 control negatives were observed. There does not appear to be any pattern in the occurrence of these false negatives. They occur equally in the EMG recordings and in the observed twitch response. An unacceptable level of variability is also seen in mm of the EMG response. The standard errors are 32% and 11% of the means for saline controls in the lidocaine and procaine tests, respectively. For the remaining analysis (Tables 36 and 37) all animals with greater than 20% control negatives were eliminated.

A second problem is that the log dose response curves are not linear. As shown in the tabulated data, the highest correlation coefficient for log-linear regression was 0.88. The animal-to-animal variability is so great that any dose relationship is obscured. In most cases there is no significant difference between doses. This is probably not a result of incorrect dose range, since the means of the observed twitch responses were between 25% and 75% negative responses.

The final problem is that even at the highest doses, about 25% of the time the animal still responds to the tactile stimulus.

The method is based on Bulbring and Wajda (1945) in which they injected 0.0125% to 0.1% of nupercaine intradermally in guinea pigs. They obtain 100% response to a tactile stimulus after the anesthetic wears off. They also have 0% response for the first 20 minutes when 0.1% anesthetic was used. Data are also shown for procaine (0.25 to 2.0% drug) with a similar general effect. For lidocaine, Camougis and Takman (1971) report complete anesthesia (12 of 12) for 70-90 minutes with 0.1 ml of 2% drug. A 0.5% solution decreased the duration to about 30 minutes. However, all of these experiments used the classical guinea pig animal model.

It was necessary, therefore, to compare the EMG method to the observed twitch response method using the classical model of the guinea pig. If this experiment, with EMG and USAIDR

TABLE 36

INTRADERMAL DOSE RESPONSE DATA

USING 0.2 ml OF PROCAINE·HCl IN SALINE

IN RABBITS

Drug Concentration %	Observation Twitch % Negative	Integrated EMG Data		
		% Negative	mm	mm-Control
0	3.5±1.8*	4.4±2.0*	619±70*	-
0.25	44±10	36±9	228±57	391
0.50	61±10	52±8	141±64	478
1.00	70±9	50±8	163±50	456
2.00	68±8	48±12	170±55	449
**r	0.88	0.61	-0.52	0.53

* Control is significantly different from each concentration of drug ($p < .05$).

** logarithmic dose relationship; does not include control (0%).

TABLE 37

INTRADERMAL DOSE RESPONSE DATA

USING 0.2 ml OF LIDOCAINE·HCl IN SALINE

IN RABBITS

Drug Concentration %	Observed Twitch % Negative	Integrated EMG Data		
		% Negative	mm	mm-Control
0	8.0±4.9*	8.0±3.4*	391±124	-
0.125	69±10	51±9	176±49	215±88
0.25	56±4	40±6	193±53	197±94
0.50	84±6	59±10	152±60	238±90
1.00	74±8	62±9	154±48	236±96
r	0.47	0.68	-0.71	0.77

* Control is significantly different from each concentration of drug ($p < .05$)

stimulator, gave data comparable to that of Bulbring and Wajda, then the rabbit is an inappropriate model for intradermal injection of local anesthetics. If the data are not improved, when the guinea pig is used, then a problem may exist with the stimulator or EMG signal pick-up and recording methodology.

2. Subcutaneous Rabbit Studies

The intradermal wheal is the standard method of determining anesthesia of nerve endings on the skin surface. However, since microcapsules cannot be injected through a 25-gauge needle, a subcutaneous route was proposed. A subcutaneous injection pilot study was performed for testing duration and extent of local anesthesia in the rabbit. Table 38 shows the duration of action of several volumes of 1.0% lidocaine-HCl in saline injected subcutaneously. These data are the observed twitch response following stimulation at the injection site.

In this experiment, anesthesia spread 1 cm from the injection site following a 0.2 ml injection volume, and 4 cm following a 0.4 ml injection. This 4 cm radius is nearly equal to the total area of the rabbit's hind quarters.

The results of this experiment were encouraging. However, concurrent with these experiments the problems with sensitivity and reproducibility of the rabbit intradermal model were discovered. Therefore, we question the validity of this approach.

Microcapsules can be injected subcutaneously, as a suspension in HPC, if a large needle is used (16-18 gauge). A very large needle (e.g. 12 gauge) can be packed with dry powder which is pushed out with a rod, as in a trocar. Preliminary data are shown in Table 38. In addition to the general problem of surface anesthesia in the rabbit, the microcapsules were observed to fall several centimeters from the point at which they were injected by trocar. The solid microcapsules could be felt under the skin. The dorsal surface of a rabbit (or guinea pig) may be a tighter surface which will allow less movement of a subcutaneously injected drug.

3. Intradermal Guinea Pig Studies

Retired breeder guinea pigs were used by Dr. Michael Gay at Northeastern University. A Centrap™ (Fisher Scientific/Biodec, Inc.) was used as the restrainer. The needle for the USAIDR device was changed from a 15 to 26 gauge needle. The 15-gauge needle broke the skin of the guinea pig, causing unwanted bleeding. The injection volume was increased from 0.20 ml to

TABLE 38

SUBCUTANEOUS INJECTION OF LIDOCAINE·HCl

IN RABBITS

Method of Injection	Total Drug (mg)	Duration of Action (minutes)	Radius of Action (cm, max)
Solution in saline			
(0.2 ml, 1%)	2	29	1.0
(0.4 ml, 1%)	4	69	4.0
(0.5 ml, 1%)	5	73	-
(0.75 ml, 1%)	7.5	81	-
Solution in HPC (0.4 ml, 1%)	4	75	1.5
Capsule Suspension in HPC (0.4 ml, 10% x 50% drug)	20	75	1.8
Trocar with dry crystals (20 mg)	20	40	2.5
Trocar with capsules (40 mg x 50% drug)	20	40	1.5

0.25 ml, to follow the method of Bulbring and Wajda more exactly. Finally, five of the six guinea pig trials using lidocaine were done in a blind manner, by injection of coded samples.

The guinea pig demonstrates an obvious twitch which is repeatable with even a small mechanical insult. With the rabbit, a gross insult was required and the mechanical skin movement had to be differentiated from a muscle twitch. With the rabbit there was no significant anesthetic logarithmic dose response (r range of 0.47 to 0.88). With the guinea pig there is an excellent logarithmic dose response (r = 0.962 to 0.9975, Tables 39 and 40. With this type of data we can also demonstrate the increased potency of lidocaine versus procaine, and the increased sensitivity of the EMG response versus the visual twitch response (Figure 36, less negative reponses with EMG than with visual observation).

Comparison of these results (Tables 39 and 40) with the rabbit data (Tables 36 and 37) indicate that the animal model, not the equipment, was the problem in repeating the data of Bulbring and Wajda (1945).

We believe the guinea pig is the more appropriate animal model because the panniculus carnosus is better developed in the guinea pig than in the rabbit (Donald Smith, Northeastern University, Director Laboratory Medicine, personal communication, Cooper and Schiller, 1975). This is a thin layer of muscular tissue just beneath or within the superficial fascia, which would respond to a cutaneous insult.

Although the guinea pig can be bled with relative ease through the orbital plexus, we cannot obtain sufficient quantities of blood at the necessary time intervals for measurement of anesthetic and metabolite circulating levels. Thus, the rabbit was used as a parallel animal model for these experiments.

Several changes in the method have been evaluated during this contract. Since subcutaneous needle electrodes are painful we later utilized miniature surface electrodes coated with EKG gel and held them in place with rayon tape (Dermacil™) or rubber dam material. We tested Copeland-Davies surface electrodes (available from Ealing Corp., Natick, Mass. and supplied by Dr. Wynkoop), and wound clips inserted as electrodes with small alligator clips and light wire for connection to the Physiograph. There was little difference observed between these electrodes but they were all superior to the needle electrodes. In order to run animals in parallel, a switch box with three electrode inputs was utilized.

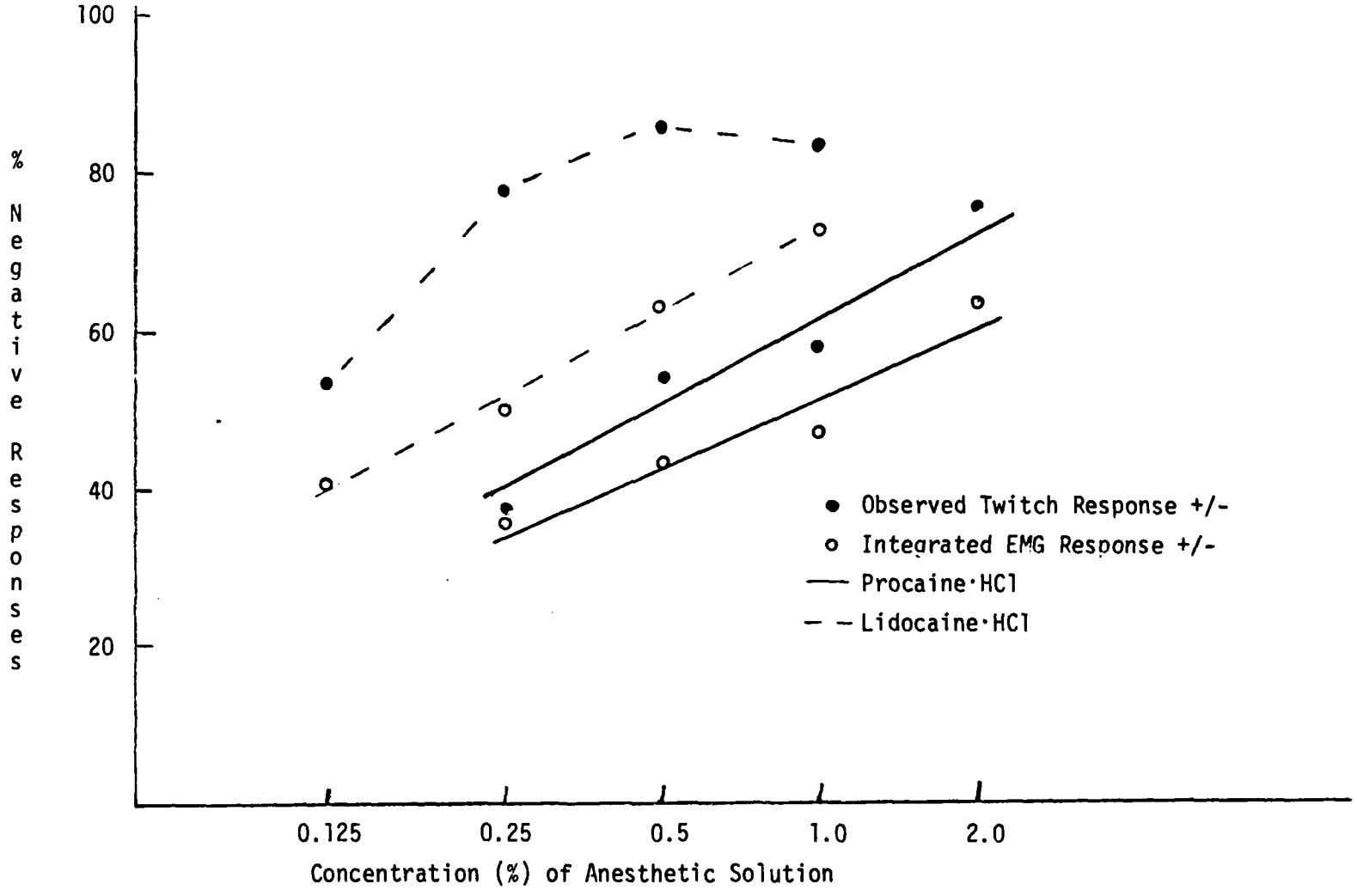


Figure 36 Intradermal Injection of 0.25 ml of Anesthetic into Guinea Pig

TABLE 39

INTRADERMAL DOSE RESPONSE DATA

USING 0.25 ml OF PROCAINE·HCl IN SALINE

IN GUINEA PIGS

Drug Concentration %	Observation Twitch % Negative	Integrated EMG % Negative	Integrated EMG mm÷Control mm (%)
0	2.1±1.3	3.1±1.5	-
0.25	36.9±7.2	35.6±5.0	66.5±2.2
0.50	53.6±7.8	42.2±2.2	55.8±17.5
1.00	57.8±7.5	46.7±4.7	29.7±4.0
2.00	74.4±6.1	62.2±7.5	24.7±6.0
* r	0.978	0.962	-0.969

* correlation coefficient, r, is based on logarithm of doses (0.25 to 2.00%)

TABLE 40

INTRADERMAL DOSE RESPONSE DATA
USING 0.25 ml OF LIDOCAINE·HCl IN SALINE
IN GUINEA PIGS

Drug Concentration %	Observation Twitch % Negative	Integrated EMG % Negative	Integrated EMG mm÷Control mm (%)
0	3.2±0.9	2.9±0.8	-
0.125	53.3±11.9	40.6±8.3	38.0±8.1
0.25	77.2±7.5	49.4±6.7	23.0±6.6
0.50	85.6±5.0	62.2±7.5	15.9±3.7
1.00	83.3±3.1	71.6±2.8	11.2±2.3
r	*	0.9975	-0.966

* too few positive responses for statistical analysis

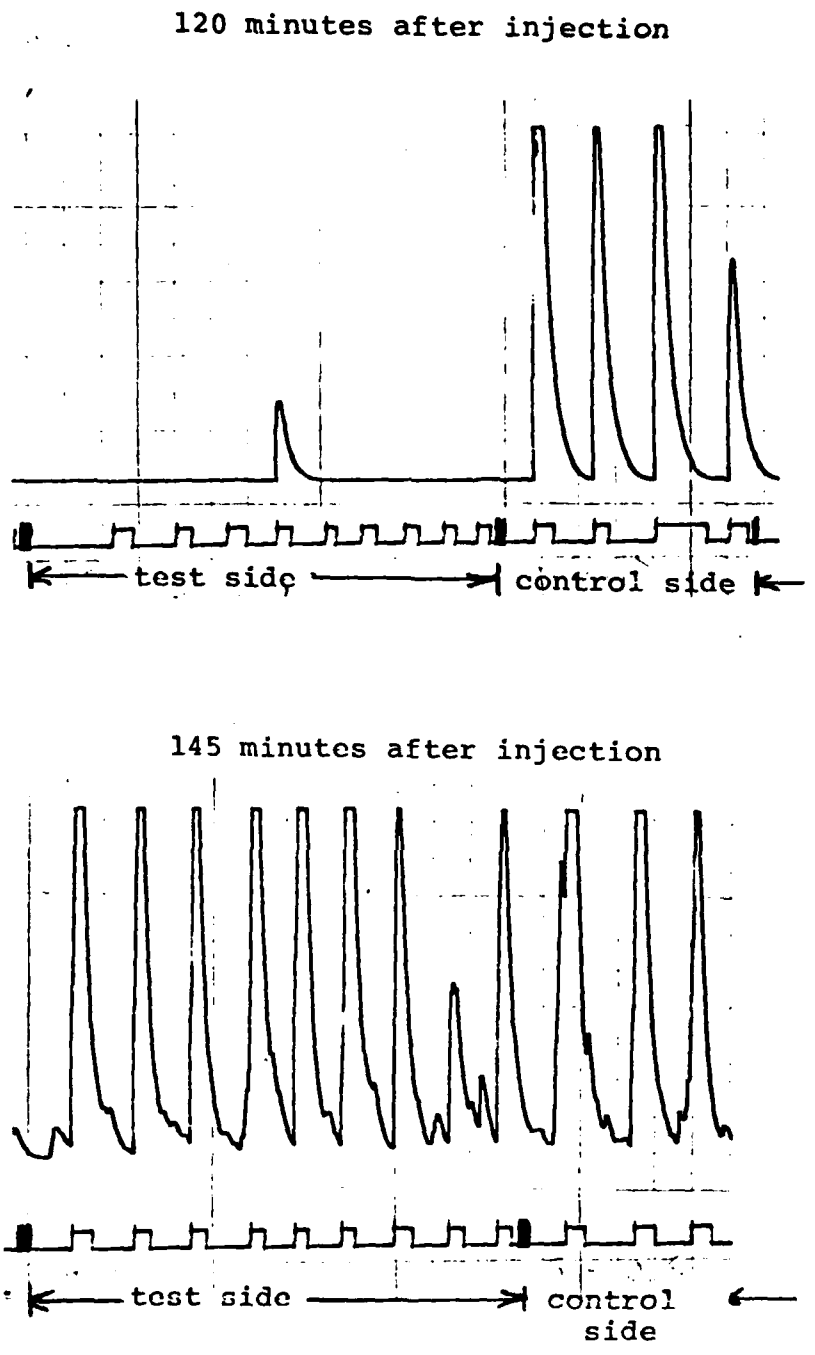


Figure 37 Integrated EMG of Guinea Pig Response (No. 8, 8/27/82) to 1 ml of 2% Lidocaine-HCl Solution Injected S.C.

To aid the injection, light ether or halothane anesthesia was utilized. In some experiments convulsions occurred which were probably the result of the combined effect of the local anesthetic and the excitation phase during emergence from the general anesthetic. This approach was therefore abandoned.

All experiments were conducted with the USAIDR stimulator and EMG recording. In addition in the intradermal studies we also performed the test by the standard method of Bulbring and Wajda (1945), in which a hand held needle is used as the stimulator and the visible twitch is observed. This stimulation is weaker and is not associated with the noise and mechanical vibration of the USAIDR stimulator. It was found to be the more sensitive system. Another stimulator was constructed by attaching a 1-inch, 26-gauge needle to a small microswitch. The microswitch closed when the stimulator needle touched the guinea pig and this activated the event marker on the Physiograph. The concept of the lateral extent of anesthesia was eliminated and only the injection site was stimulated. Representative data, Figure 37, obtained using the USAIDR stimulator and lidocaine-HCl solution shows the data output when the system is functioning properly.

We compared 1% lidocaine-HCl in solution and microcapsules (06-1-50, 150-212) as subcutaneous and intradermal injections. The subcutaneous injections were performed first with 0.5 ml of vehicle (5 mg). The results were variable. The most useful analysis was of the total number of anesthetic responses. Responses were elicited at approximately 5 minute intervals, and anesthesia was never observed after 65 minutes; hence, we assumed that there were 65 possible negative responses (13 x 5 = 65 responses/group). The data are presented in Table 41. Microencapsulated lidocaine provided less anesthesia than the solution.

Next, a dose response study comparing lidocaine-HCl microcapsules and solutions was carried out. Each animal received four intradermal injections and three to five wheals were tested at each concentration. For the solution there is a dose response relationship for both mean number of anesthetic responses and duration of anesthesia. However, no advantage was observed between the microcapsules and the solution. Necrosis was observed at the injection site when more than 5 mg of drug was injected. Furthermore, it was not possible to place more than 5 mg of lidocaine-HCl as a microcapsule suspension into a single intradermal wheal.

During these experiments we suspected that intradermal injection of microcapsules might not deliver the expected quantity of material. In three experiments the residual anesthetic in the syringes was measured and only 23% of the microencapsulated anesthetic was injected. Apparently, the intradermal

TABLE 41

INJECTION OF 1% LIDOCAINE-HCl IN GUINEA PIGS

	Subcutaneous (0.5 ml, 5 mg)		Intradermal (0.25 ml. 2.5 mg)			
	Soln.	M.C.	Soln.		M.C.	
	USAIDR EMG	USAIDR EMG	USAIDR EMG	Needle Twitch	USAIDR EMG	Needle Twitch
Total negative responses	10/65	7/65	9/65	37/65	5/65	15/65
Animals with negative response	5/5	3/5	4/5	5/5	3/5	5/5
Ave. time of anesthesia (minutes)	-	-	-	51±10	-	15±10

space acts as a sieve, preventing the passage of microcapsules from the needle. Other suspending media for the microcapsules were tried, without a noticeable improvement. Injecting saline to form the wheal prior to injecting the microcapsules was unsuccessful because the volume of the initial wheal could not be returned to the syringe. The syringe became plugged with the dermal tissue. We know that the syringe delivery of microcapsules was not a problem with intraperitoneal injections in mice and it could be overcome with intramuscular injections in rabbits. We then measured the quantity of material left in the syringe, and calculated the amount injected intradermally. However, this did not significantly alter our basic conclusions. It appears that a sufficient quantity of microencapsulated lidocaine cannot be injected intradermally.

Since etidocaine is four times more potent than lidocaine, this local anesthetic agent was tested in the next series of experiments. The results are presented in Table 42. Preparation of etidocaine-HCl microcapsules, etidocaine-HCl solution, and a suspension of etidocaine (base) were tested. Material was suspended or dissolved in the HPC vehicle and injected intradermally with an 18 gauge needle and the duration of local anesthesia was determined. When log dose response curves were constructed based on the actual administered dose, the solution and free base suspension appeared to be more potent by approximately an order of magnitude than the microcapsules. However, the full dose of the microencapsulated material is not available due to the sustained release. Based on the in vitro release data the actual amount of drug released from the microcapsules during the period of anesthesia was calculated. The response was then plotted against the log of the amount of drug released for the solution and for the 11-2-20 (74-106 micron) microcapsule preparation (Figure 38). Similar plots for the other preparations are not shown.

In this analysis the longest duration for the least drug was observed for the 11-2-20 (74-106 micron) microcapsules. Slightly shorter durations were observed for the 11-2-30 (74-106 micron) preparation which has a smaller initial release or burst. The 11-2-30 (39-75 micron) microcapsules which show the fastest in vitro release characteristics gave a shorter duration of action.

These results are consistent with a sustained release and action with microencapsulated etidocaine. The 11-2-20 (74-106 micron) microcapsules were approximately two times more potent than the solution (Figure 38). Unfortunately, the amount of material which can be injected intradermally is limited. In addition, at the dose levels employed in these studies, both lidocaine-HCl and etidocaine-HCl, both as solutions and microcapsule suspensions, caused tissue necrosis at the injection site.

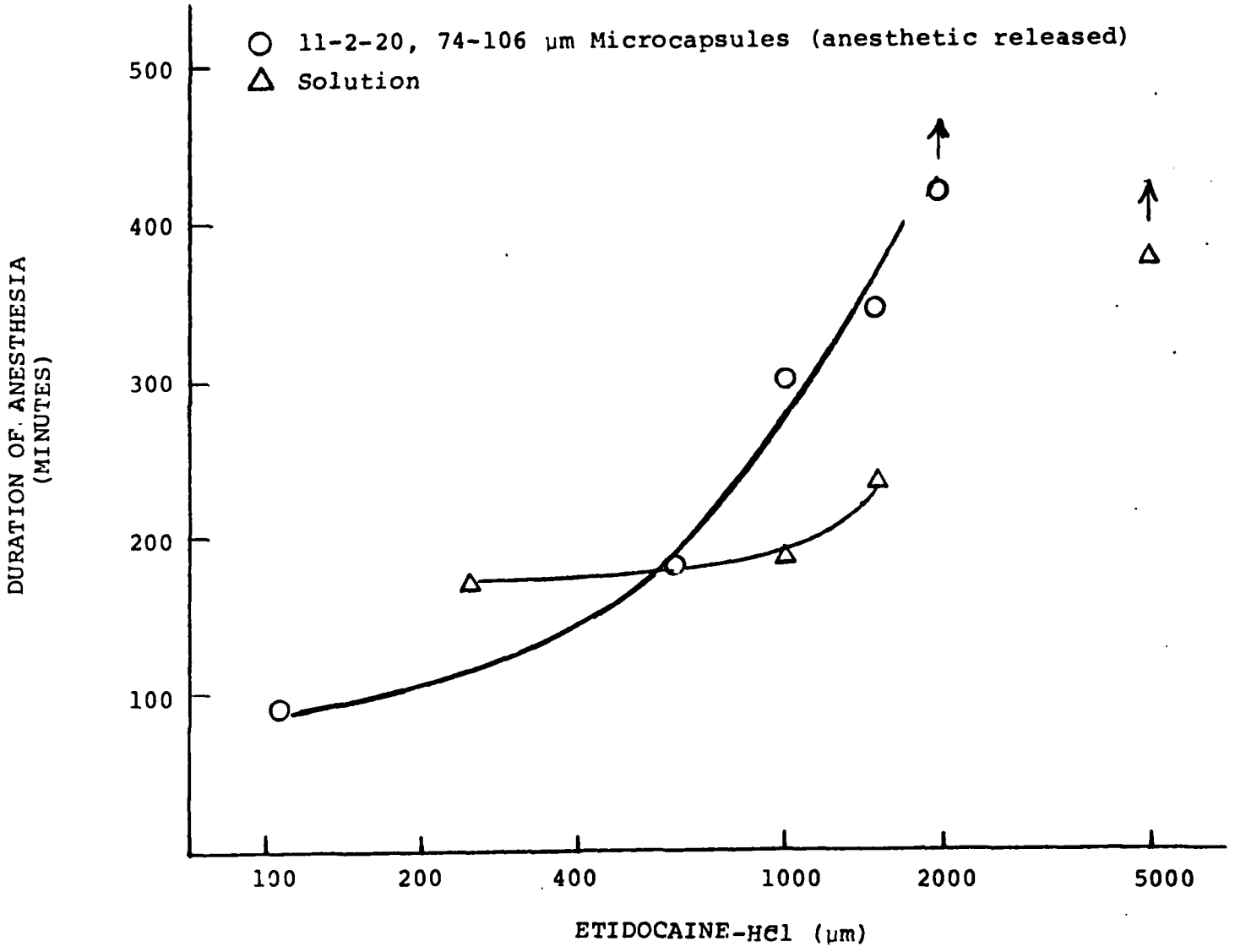
TABLE 42

ETIDOCAINE INTRADERMAL STUDY IN GUINEA PIGS

Theoretical Drug Dose (mg)	Actual Drug Dose (mg)	# An.	Duration (minutes)	In Vitro % Release	µg Drug Released*	µg Drug Released/min. of Anesthesia
Microcapsule Preparation 11-2-30, 74-106						
0.25	0.215	2	90	40	86	0.95
1.0	0.448	2	135	45	202	1.50
1.5	0.340	2	150	47	160	1.07
2.0	0.980	2	217	56	549	2.50
Microcapsule Preparation 11-2-20, 74-106						
0.25	0.24	2	90	45	108	1.0
1.0	1.0	2	180	62	620	3.4
1.5	1.3	3	300	80	1040	3.47
2.0	1.8	2	300 to >390	80 to >86	1440 to >1548	4.8 to <4.0
2.5	2.3	2	>420	>86	>1978	<4.7
6.7	6.0	2	>450	>89	>5340	<11.9
Microcapsule Preparation 11-2-30, 38-75						
0.25	0.25	2	82	54	135	1.65
1.0	1.00	2	150	76	760	5.07
1.5	1.50	2	210	86	1290	6.14
Solution						
0.25	0.25	2	170	100	250	1.47
1.0	1.0	2	187	100	1000	5.78
1.5	1.5	4	236	100	1500	7.7
5.0	5.0	1	>375	100	5000	< 13.3
Etidocaine Base (Suspension)						
0.25	0.25	2	30	100**	250	8.3
1.0	1.0	1	230	100**	1000	4.3
1.5	1.5	2	215	100**	1500	6.7
2.0	2.0	2	315	100*	2000	6.4

* Actual dose x % in vitro release during duration of anesthesia
** Assume 100% available

Figure 38 Duration of Anesthesia Using Soluble and Microencapsulated Etidocaine-HCl in Guinea Pig Intradermal Wheal



4. Rat Sciatic Nerve Block

Due to limitations imposed by the intradermal guinea pig model a pilot study was carried out using a different model system. Etidocaine-HCl solutions and etidocaine-HCl microcapsule suspensions were injected into rats to achieve a block of the sciatic nerve. The end point of these experiments is an obvious opening of the toes on the affected leg and an ability of the rat to walk normally again. This experimental block of a major sheathed nerve is a classical local anesthetic model. Although anesthesia of wounds would be considered surface anesthesia of exposed nerve endings, this nerve block experiment does lead to an anesthetic duration value. The results of the first experiment are shown in Table 43. An 18 gauge needle was used to inject 0.25 ml of 1% etidocaine-HCl as a solution or microcapsule suspension into the tissue area near the sciatic nerve. In this experiment the anesthetic was injected into the region of the sciatic foramen using the trochanter of the femor and the iliac crest as guides. The results of this pilot study suggested that the rat sciatic nerve model would provide a definitive test of the hypothesis that sustained release preparations of local anesthetic agents could provide prolonged anesthesia without toxicity.

In the next series of experiments, etidocaine-HCl microcapsules (11-4-30, 150-212 micron) were suspended in the HPC vehicle and the pure drug was dissolved in the vehicle immediately before use. The material was injected with a 1-1/2-inch 18-gauge needle into the region of the sciatic nerve in the thigh of the rat. In this experiment the injection was made between the thigh muscles of the rat and the popliteal surface of the femor. A larger volume of anesthetic was reproducibly injectable into this space. Generally, a 0.5 ml injection volume was used except for the highest concentrations of microcapsules. The animals were observed for splayed digits and paw paralysis as well as convulsions and death. In addition to determining the duration of anesthesia, the percent of animals at each dose that showed anesthesia for 12, 24, and 36 hours was calculated.

Due to the small number of animals (5 - 6 per group), this data and the other quantal data were analyzed by the double integration method of Dragstedt and Lang (1928) and then plotted on semilogarithmic - probability paper to determine median anesthetic effective dose (ED-50). The experiment was carried out twice, first with older 450 gram rats and then with 300 gram rats. The data for anesthesia and convulsions were the same in both studies. However, only the older rats died in the dose range studied. The most probable explanation is that the older rats had more compromised respiratory systems and could not cope with the convulsions as well as the younger animals.

TABLE 43

DURATION OF RAT SCIATIC NERVE BLOCK FROM SOLUTION AND SUSPENSION OF ETIDOCAINE·HCl

	Anesthetic Duration (minutes)	
Rat No.	1% Solution	1% as Suspension*
1	68.4	138.5
2	49.1	255.5
3	73.7	103.3
4	52.1	200.1
5	111.5	178.0
Ave.	71.0	175.1
S.D.	25.0	58.3
S.E.M.	11.2	26.1
t	3.67	
p	<0.01	

* Run 11-2-30, 106-300 micron microcapsules

In Figure 39 the toxicity data is presented. The 50% convulsive dose (CD-50) was 48 mg/kg for etidocaine solution and 322 mg/kg for the microcapsules. In the older rats the LD-50 was 78 mg/kg for the solution and 338 mg/kg for the microcapsule suspension. The increase in safety of the microencapsulated etidocaine supports the results obtained in the mouse toxicity studies.

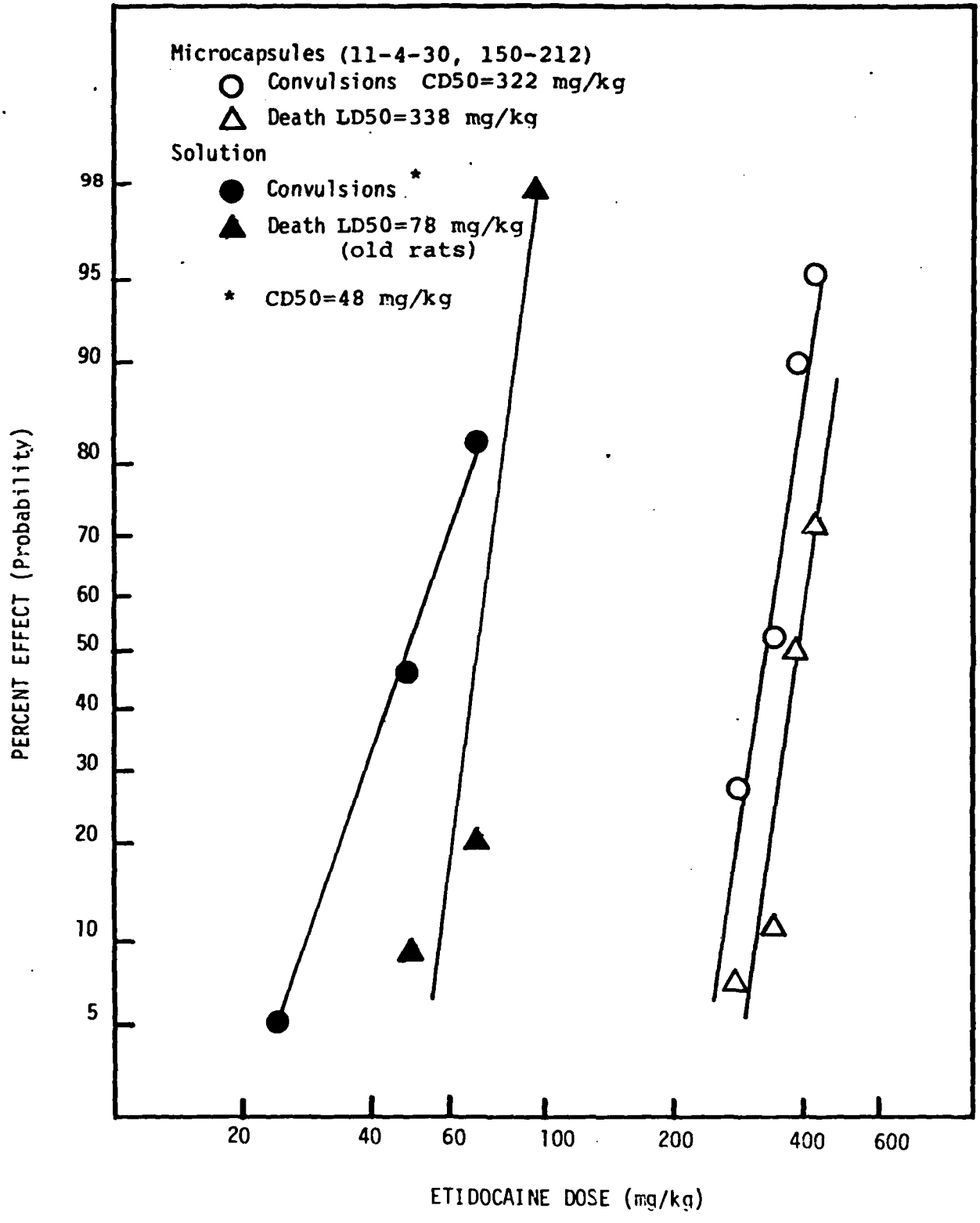
It should be noted that while the microcapsule suspension took slightly longer to act and showed greater variability in onset time than the solution over the entire dose range, there were no significant differences in either the time of onset of initial partial local anesthesia or complete anesthesia between the solution and suspension. The initial signs of local anesthesia were observed in 22.4 $\pm$4.0 seconds for the solution and 30.5 $\pm$10.8 seconds for etidocaine microcapsules. Complete nerve block was established in 67.0 $\pm$11.4 and 99.1 $\pm$20 seconds for solution and suspension, respectively.

The duration of sciatic nerve block for etidocaine solution and microcapsule suspension is shown in Figure 40. There is a plateau in duration of anesthesia induced by the solution at about 30 hours and this requires a dose of 48 mg/kg. Microencapsulated etidocaine provided much longer durations--almost 90 hours. It is apparent that the microcapsules are less potent based on amount administered than the solution. This is to be expected as a consequence of sustained release. However, a duration of 24 hours or greater was possible with the solution, only at or above the CD-50 drug dose for solutions.

If the percent of animals reaching a criterion is plotted against the log of the dose, a median effective dose (ED-50) can be calculated. This data are shown in Table 44 and Figures 41 and 42. While only one animal demonstrated local anesthesia for 48 hours following administration of the solution, an ED-50 of 160 mg/kg was observed for microencapulated etidocaine. For 48 hours of local anesthesia by microcapsules the median therapeutic index, relative to convulsions, (CD-50/ED-50) was 2.01. For solutions of etidocaine-HCl, the ED-50 for 36 hours of anesthesia was 100 mg/kg and for 24 hours duration it was 64 mg/kg. In each case the median therapeutic index relative to convulsions is less than one. For 12 hours of local anesthesia the ED-50 for the solution was 40 mg/kg, for a therapeutic index of 1.20. The data for 24-hour anesthesia are shown in Figure 43.

Therefore, while etidocaine is a long acting local anesthetic agent, its systemic toxicity places a limit on the maximum duration that can be achieved. For a duration greater than or equal to 12 hours the dose required will induce convulsions in about 50% of the animals. On the other hand, microencapsulated etidocaine can provide 48 hours of local anesthesia for 50% of

Figure 39 Rat Sciatic Nerve - Toxicity Data



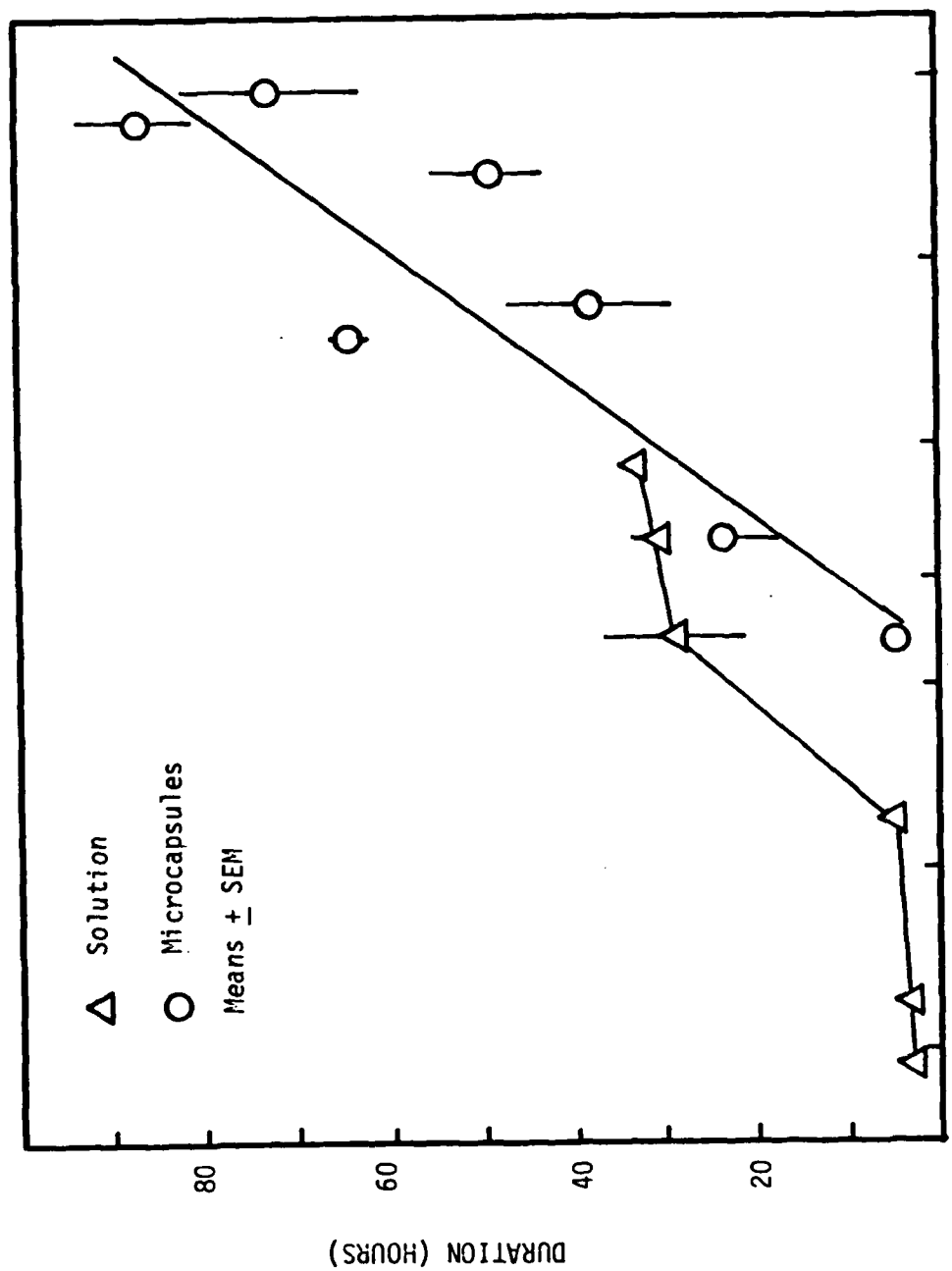


Figure 40 Duration of Rat Sciatic Nerve Block

TABLE 44

QUANTAL DATA OF RAT SCIATIC NERVE EXPERIMENT
(Anesthesia Times, Convulsions and Deaths)

Rat Dose mg/kg Solution	% Death*	% Convulsive	% Anesthetized After Specified Hours 12	24	36	48
12	0	0	0	0	0	0
24	0	5	0	0	0	0
48	5	47	83	20	8	0
70	9	82	93	62	12	20
92	54	100	100	100	43	-
189	100	100	-	-	-	-
Microcapsules**						
70	0	0	31	33	9	20
153	0	0	60	47	50	38
169	0	0	100	76	64	73
279	3	28	-	85	73	-
338	5	53	100	100	100	100
377	31	90	100	100	100	100

* Both sets of rats combined
** 11-4-30, 150-212, dose as Etidocaine-HCl

Figure 41 Anesthesia, Convulsions and Death with Etidocaine-HCl Microcapsules in Rat Sciatic Nerve Experiments

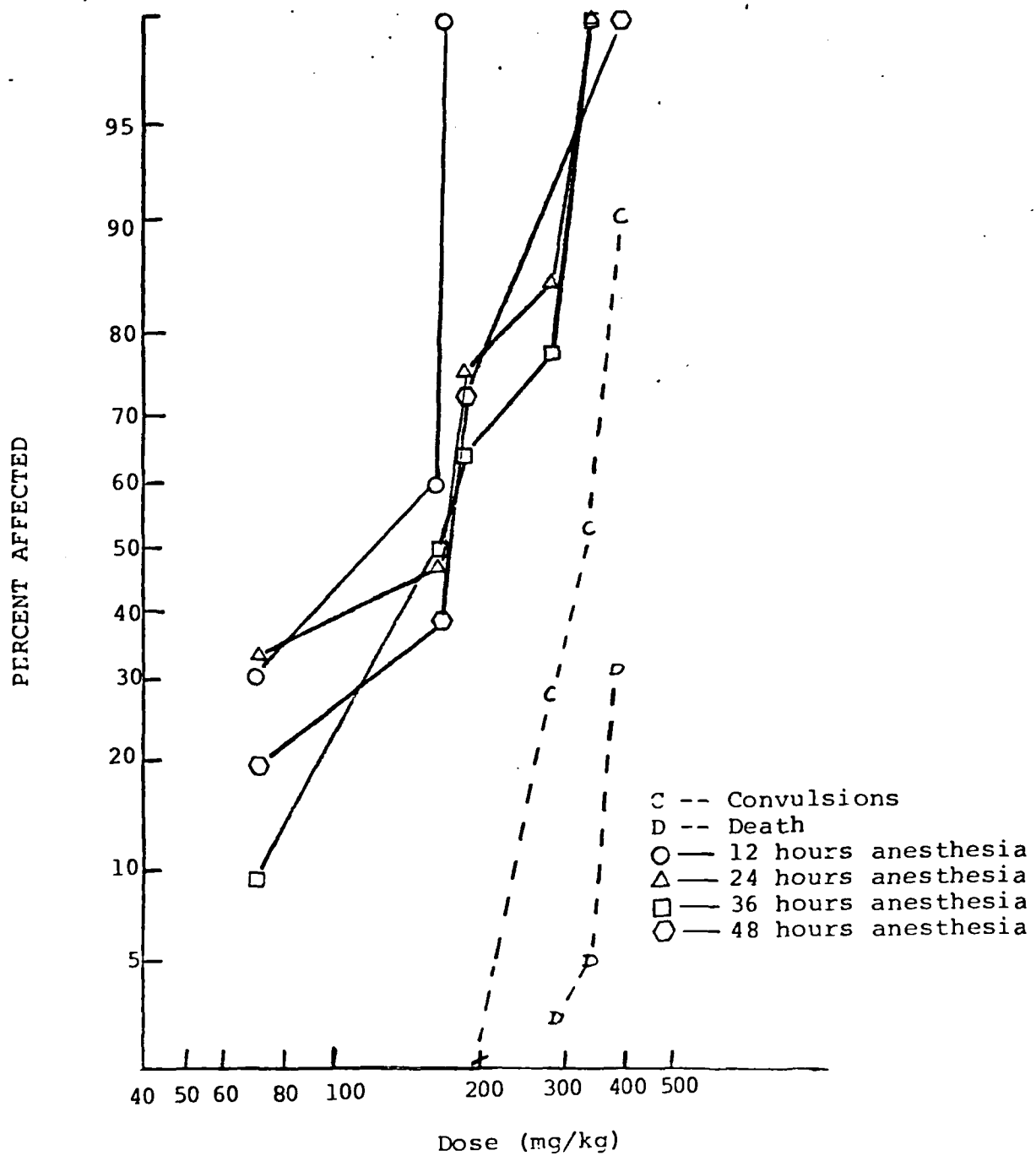
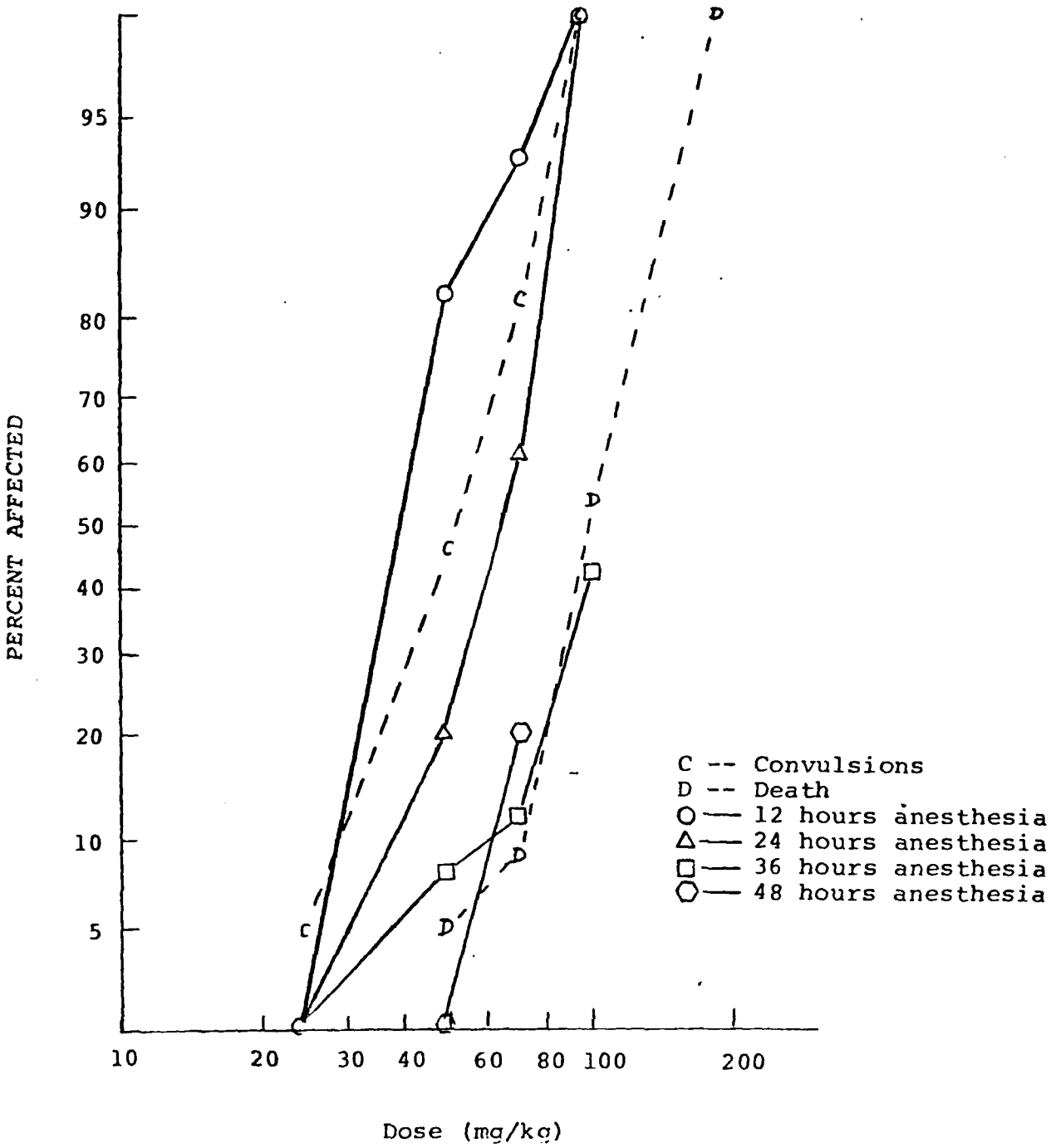


Figure 42 Anesthesia, Convulsions and Death with Etidocaine-HCl Solutions in Rat Sciatic Nerve Experiments



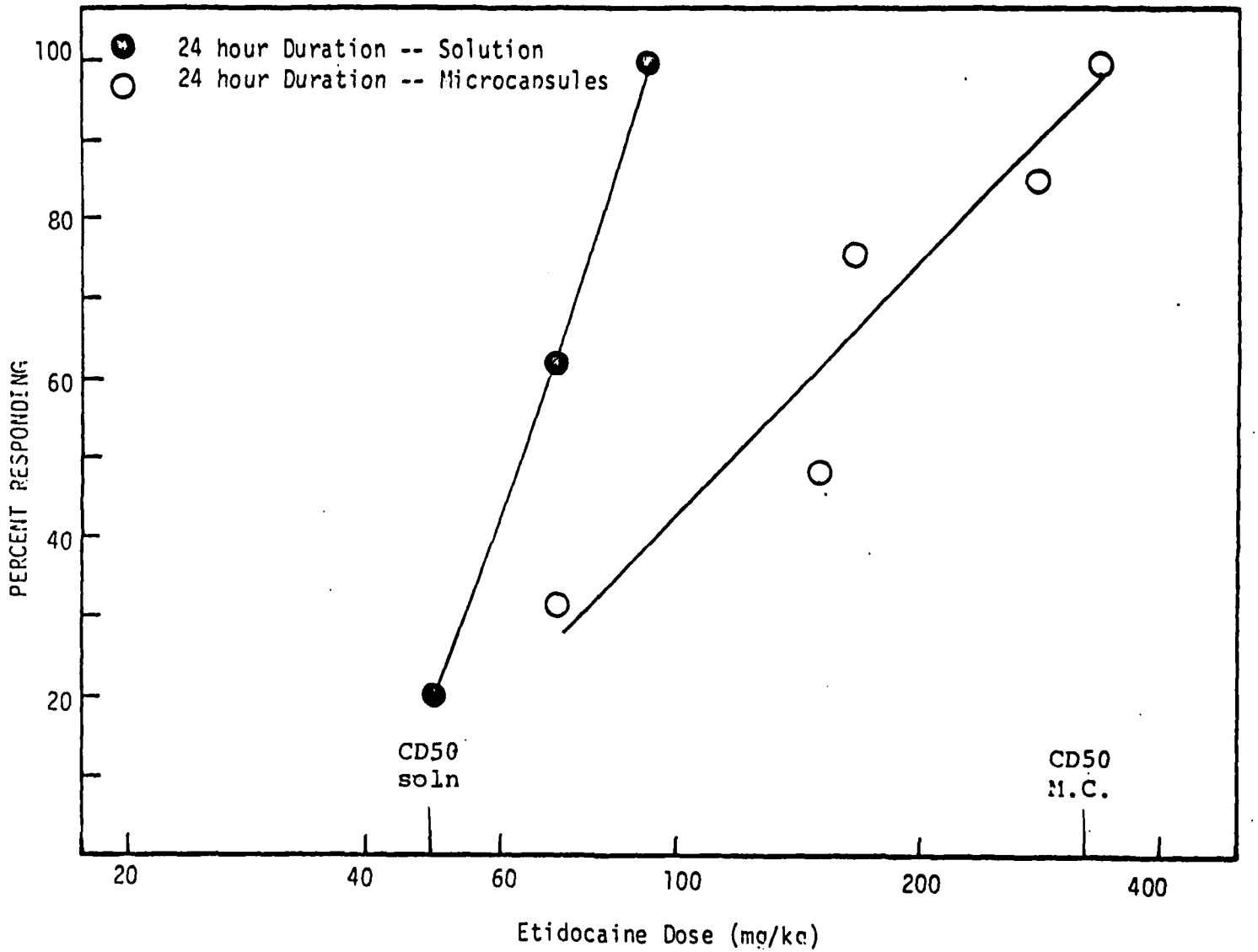


Figure 43 One Day Duration of Rat Sciatic Nerve Block for Etidocaine Solutions and Microcapsules

the rats at a dose which does not induce convulsions. This is an increase of greater than 4-fold in effective duration of anesthesia.

Epinephrine is frequently mixed with solutions of local anesthetics to slow systemic absorption. We investigated the effect of adding epinephrine (1:200,000) to etidocaine solutions and suspensions of etidocaine microcapsules and combinations of solution and microcapsules. The epinephrine significantly reduced the toxicity of etidocaine solutions and slightly increased the duration of action (Table 45). Suspending the microcapsules in a solution of etidocaine (10 mg/kg) slightly increased the duration of action but at the expense of significantly higher toxicity. Epinephrine added to a suspension of microcapsules had no discernible effect on toxicity and may have slightly reduced the duration of action. Finally, epinephrine added to a suspension of microcapsules in etidocaine solution slightly increased the duration action at low doses, had little effect at higher doses, and did not affect toxicity. Overall, these combinations did not appear to have any therapeutic merit.

TABLE 45

Effect of Addition of Epinephrine and Soluble Etidocaine to Suspension of Microcapsules.

Dose/Treatment	Percentage of Rats Anesthetized for 12 hr.	24 hr.	36 hr.	48 hr.	Convulsions %	Death %
Solution mg/kg & Epinephrine (1:200,000)						
80	0	0	0	0	0	0
189	100	100	--	--	80	60
Microcapsules mg/kg & Epinephrine (1:200,000)						
47	--	40	0	0	0	0
70	20	20	20	20	0	0
189	60	20	0	0	0	0
278	100	20	20	--	0	0
Microcapsules mg/kg & Solution (10mg/kg)						
47	--	0	0	0	0	0
94	--	0	0	0	0	0
189	100	100	--	--	80	60
Microcapsules mg/kg & Epinephrine (1:200,000) & Solution (10mg/kg)						
47	--	20	20	20	0	0
94	0	0	0	0	0	0
189	100	10	--	--	20	0

V. LITERATURE CITED

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VI. APPENDIX

A. Presented Paper

B. Diffusion of Microcapsules

A. Presented Paper

Data collected on this contract was presented at the Twelfth Annual Meeting of New England Pharmacologists. This meeting was held at the Marriott Hotel in Newton, Mass., on February 4-5, 1983.

The title, authors, and associations were listed as:

SUSTAINED RELEASE, MICROENCAPSULATED, LOCAL ANESTHETIC AGENTS

D.L. Williams, D.E. Creeden, E.S. Nuwayser, J.R. Wynkoop, L. Kazyak, D. Hadjilambris, M.H. Gay. BIOTEK, Inc., Woburn, MA 01801; U.S. Army Institute of Dental Research, Washington, D.C., 20012; Walter Reed Army Institute of Research, Washington, D.C., and Section of Pharmacology, Northeastern University, Boston, MA 02115.

The poster data is reproduced in the following pages. The message was:

1. Abstract
2. Variation of drug delivery rate by choice of anesthetic

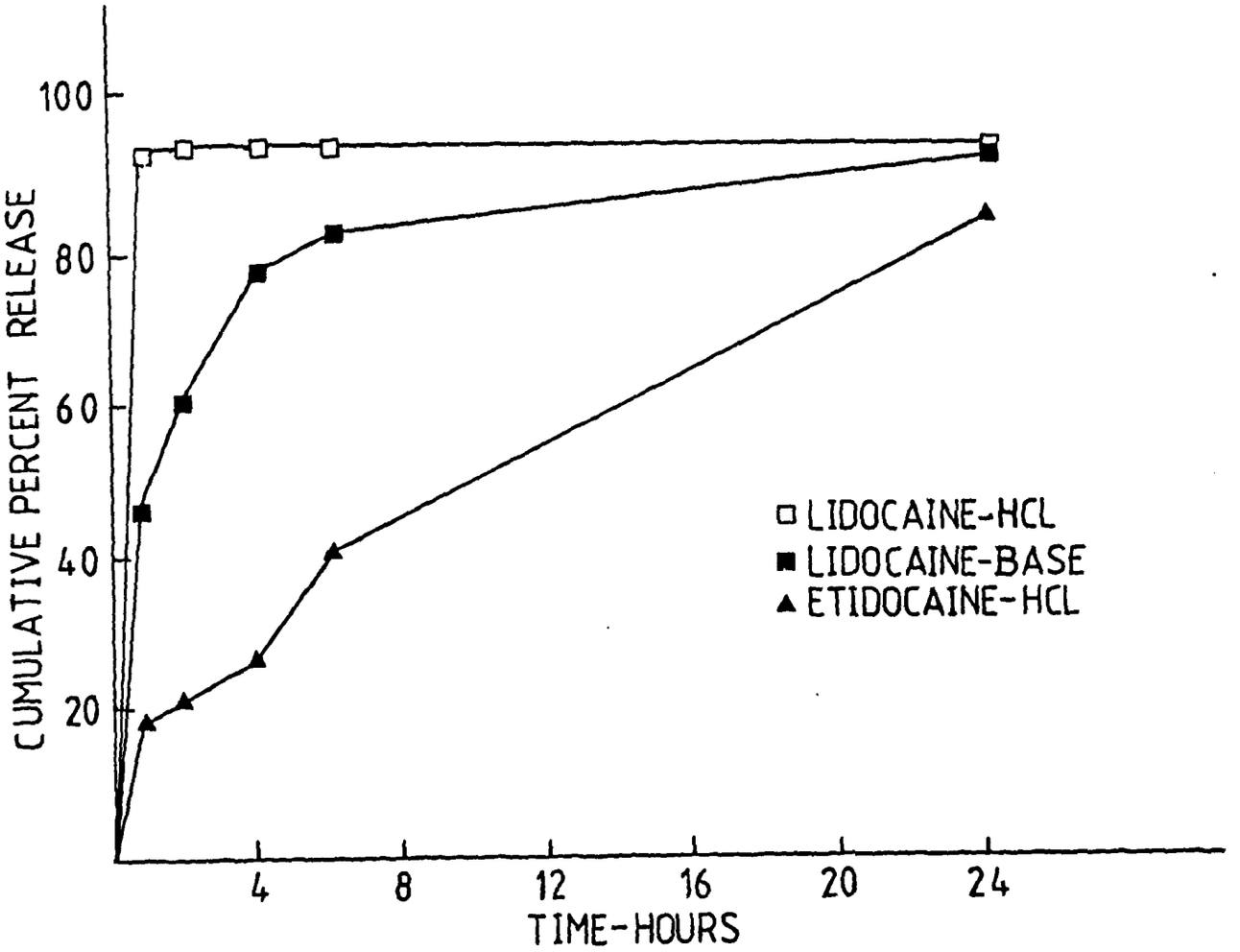
Solubility:	Lidocaine-HCl in water	570 mg/ml
	Lidocaine (base) in water	3.5
	Lidocaine-HCl in buffer	240
	Etidocaine-HCl in buffer	24
	Bupivacaine-HCl in buffer	39

3. Drug release slower from larger microcapsules.
4. Drug release slower from microcapsules with more polymer coating.
5. Drug release of microcapsules selected for <u>in vivo</u> studies.

6. Circulating levels of anesthetics shows evidence of sustained release.
7. Lower systemic toxicity of microencapsulated anesthesia.
8. Lower local toxicity of microencapsulated anesthetics.
9. Increased duration of anesthesia by encapsulated anesthetic.

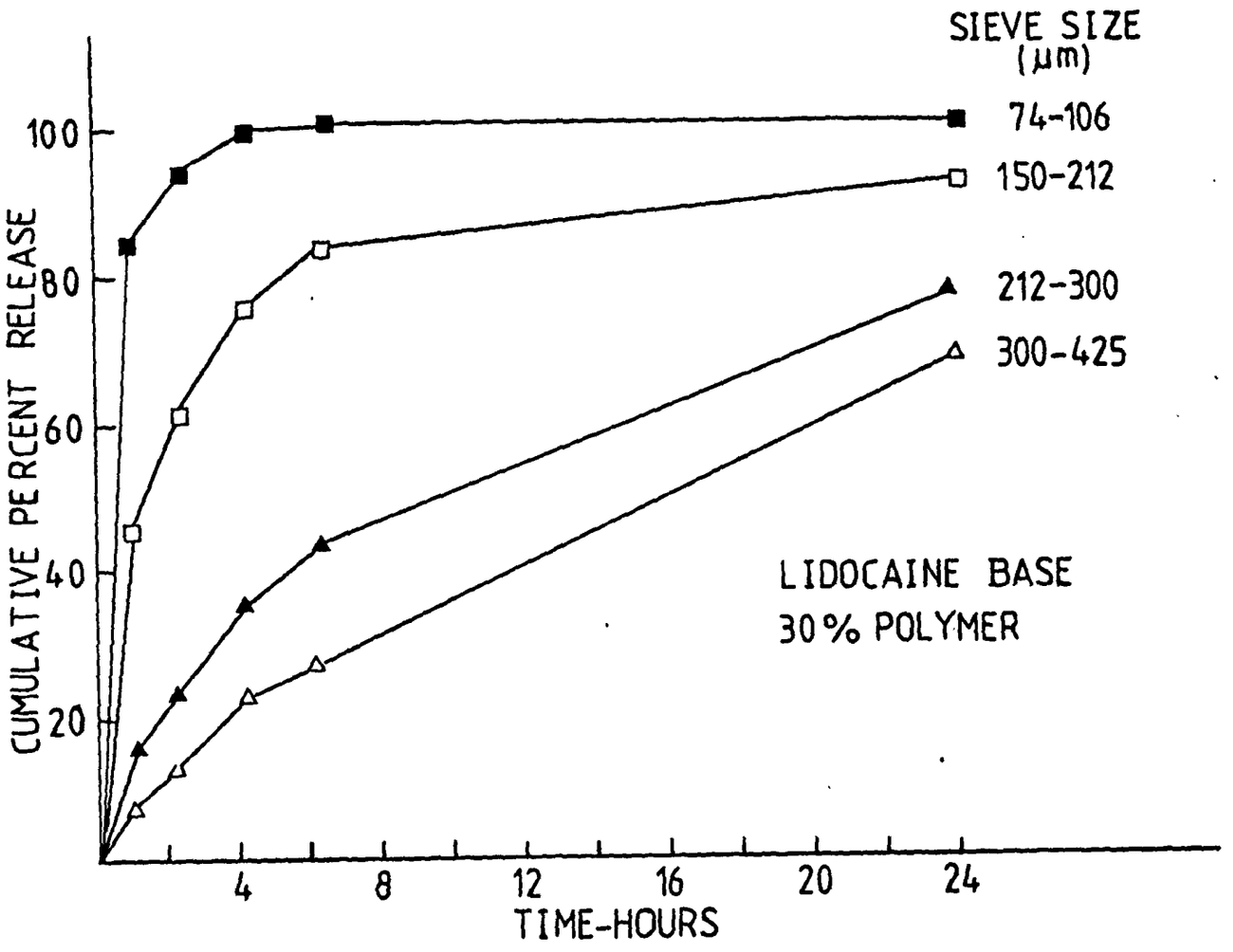
ABSTRACT

Microencapsulated local anesthetic agents were prepared by coating crystals with polylactide using the Wurster air suspension method. In vitro rate of release from microcapsules was proportional to the solubility of drug and inversely proportional to both the size of microcapsules and thickness of coating. Both LD-50 and median convulsant dose (CD-50) in mice were higher for suspensions of microcapsules than for solutions; 4.1, 5.0, and 6.7 times for etidocaine-HCL (ETIDO.), lidocaine-HCL (LIDO.) and bupivacaine-HCL respectively. Following I.M. injection in rabbits of 3 ml of 4% LIDO. in solution a toxic peak blood level (5.6 μg/ml) was observed at 10 min. while following microcapsules a lower peak (0.9 mg/ml) was observed at 1 hr. From 2 to 6 hrs. no differences in blood levels between solution and microcapsules were observed. Rabbit serum CPK, a measure of tissue damage, was higher following I.M. injection of 3 ml of 4% LIDO. solution than for a suspension of microcapsules. Duration of rat sciatic nerve block (0.25 ml, 1% ETIDO.) was greater for suspension of microcapsules 175 (± 26 SE) min. than for a solution, 71.0 (± 11.2 SE) min. These preliminary data suggest that microencapsulated local anesthetic agents give sustained release, lower toxicity, and longer duration than solutions. (Supported by contract #DAMD17-80-C-0110 and DAMD17-81-C-1195).



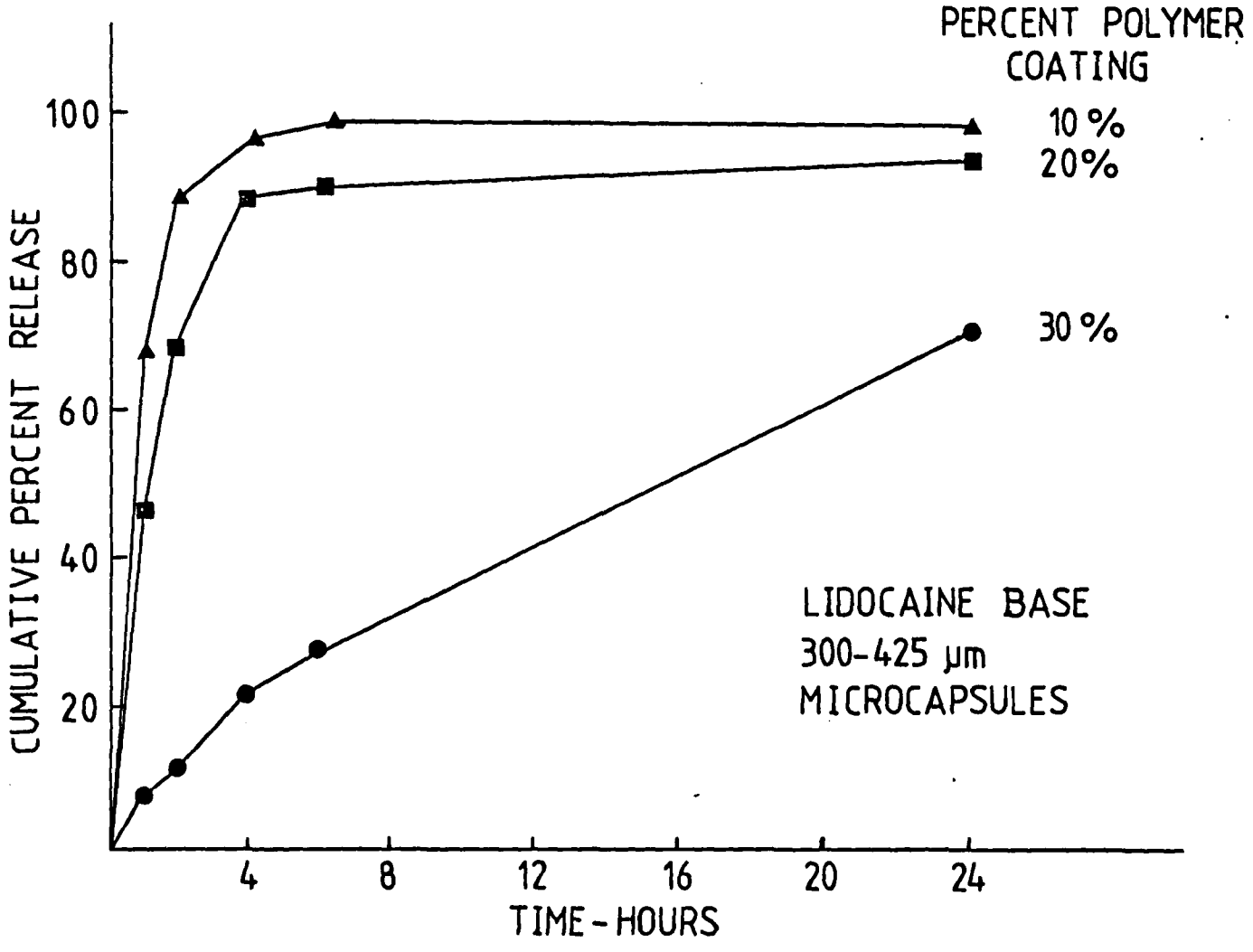
RELEASE OF VARIOUS AMIDE ANESTHETICS INTO PH 7.4 BUFFER

(30% Polymer Coating, 150-212 μm Sieve Size)

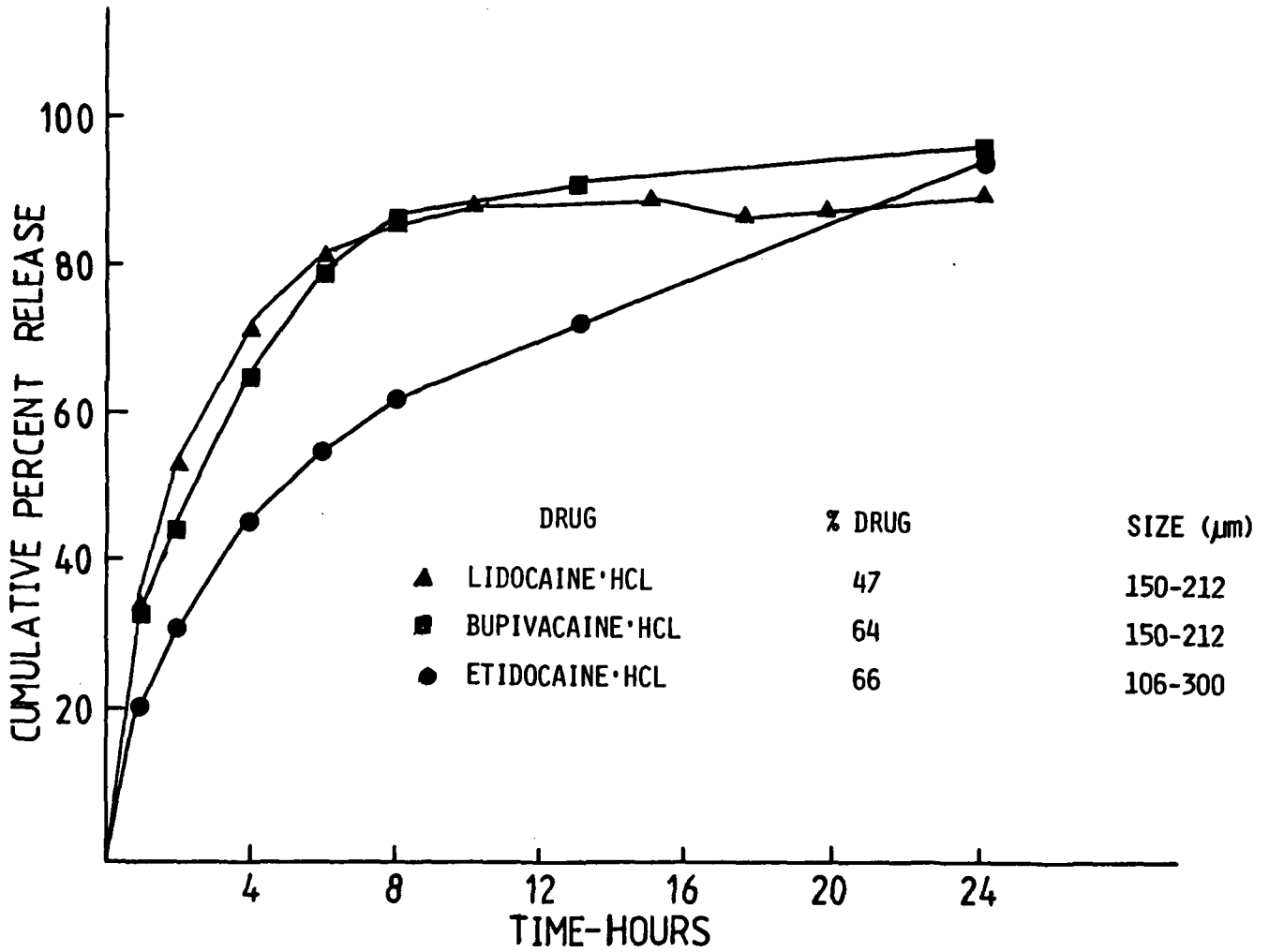


RELEASE OF ANESTHETIC FROM MICROCAPSULES INTO PHOSPHATE BUFFER

AS A FUNCTION OF SIZE

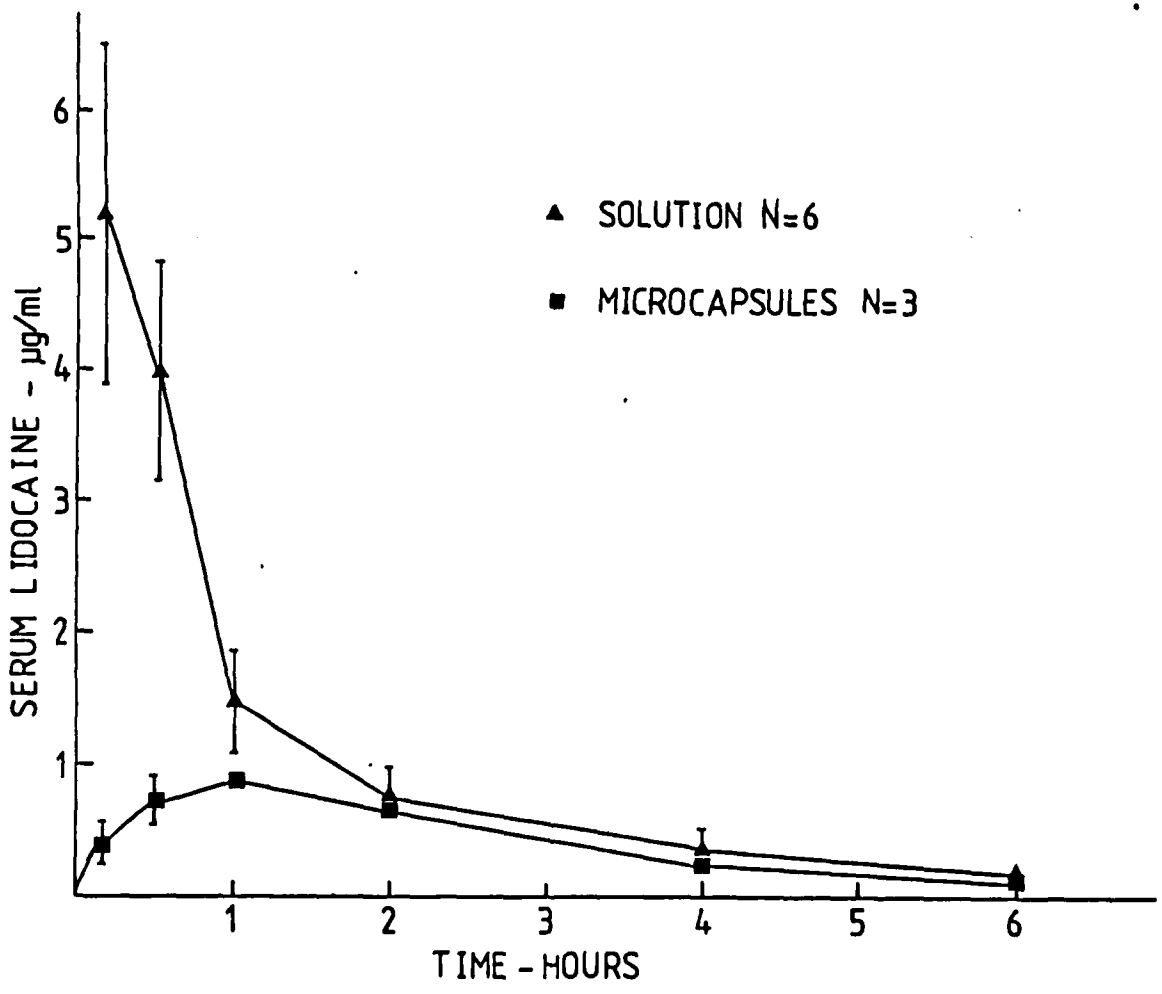


RELEASE OF ANESTHETIC FROM MICROCAPSULES INTO PHOSPHATE BUFFER AS A FUNCTION OF POLYMER COATING



MICROENCAPSULATED ANESTHETIC AGENTS SELECTED FOR IN VIVO STUDIES

(In Vitro Release Into pH 7.4 Phosphate Buffer)



SERUM LEVELS OF LIDOCAINE AFTER INJECTION OF 120 MG OF LIDOCAINE·HCL
AS SOLUTION OR MICROCAPSULE SUSPENSION
(3 ml of 4% Lidocaine·HCl as Either a Solution or as a Suspension of
Microcapsules of 50% Drug and 150-212 µm Were Injected I.M. In Rabbits)

LOWER SYSTEMIC TOXICITY

RELATIVE TOXICITY OF ENCAPSULATED AND FREE ANESTHETICS

		LD 50	CD 50	LD50/CD50
LIDOCAINE	Solution	181±12	92± 7	1.99
	Microcapsules	838±78	488±42	1.72
	M.C./Soln	4.6	5.3	
ETIDOCAINE	Solution	62± 7	47± 4	1.32
	Microcapsules	260±34	191±21	1.36
	M. C./Soln	4.2	4.0	
BUPIVACAINE	Solution	68± 5	42± 6	1.63
	Microcapsules	453±40	280±44	1.62
	M.C./Soln	6.7	6.7	

The LD 50 and convulsant dose (CD 50) were determined for solutions and suspensions of microcapsules following I.P. injection into CD-1 mice. Data is expressed as the mean dose of the drug hydrochloride in mg/kg ± SEM.

LOWER LOCAL TOXICITY

SERUM LEVELS OF CREATINE PHOSPHOKINASE AFTER INJECTION OF 120 MG OF LIDOCAINE·HCL AS SOLUTION OR MICROCAPSULE SUSPENSION

(3 ml of 4% Lidocaine·HCl as either a Solution or as a Suspension of Microcapsules of 50% Drug and 150-212 um Were Injected I.M. in Rabbits)

	PRE-INJECTION	24 HOURS POST - INJECTION
SOLUTION	345± 56	4096±542
MICROCAPSULES	377± 53	1868±372
VEHICLE	527± 80	2228±430

Units are I.U./liter, Mean ± SEM, N=6

INCREASED DURATION

DURATION OF RAT SCIATIC NERVE BLOCK FROM SOLUTION AND MICROCAPSULE SUSPENSION OF ETIDOCAINE·HCL

ANESTHETIC DURATION IN MINUTES

0.25 ML OF 1% ETIDOCAINE·HCL

SOLUTION	MICROCAPSULE SUSPENSION
71.0±11.2	175.1±26.1

$p < 0.01$

Microcapsules of 30% coating level, 106-300 μm

B. DIFFUSION OF MICROCAPSULES

DATE	NB.Pg	DRUG	CODE	WEIGHT	MIN	ACTUAL	SIZE LOW	SIZE HIGH	% DRUG RELEASE 0	1	2	4	6	12	24	48	72	96	120	144	TOTAL	STORAGE TEMP	HUMID	LIGHT	TIME YRS.	DATE STORED
		BUPI-HCl	06.3.50		50	86.4	75	106	10	36.5	41.3	45.9	49.3		57.5		64.8									
		BUPI-HCl	06.3.50		50		106	150	10	55.3	71.2	81.7	88.8		97.2		109.4									
		BUPI-HCl	06.3.50		50		150	212	10	-11.0	-13.7	-16.0	-17.0		-23.7		-35.2									
		BUPI-HCl	06.3.40		60	86.2	75	106	10	87.5	88.2	89.5	90.6		90.2		90.3									
		BUPI-HCl	06.3.40		60		106	150	10	44.6	50.7	58.2	63.8		78.8		92.0									
		BUPI-HCl	06.3.40		60		150	212	10	10.2	12.1	14.9	17.7		-23.9		-41.3									
		BUPI-HCl	06.3.30		70	90.4	38	75	10	54.2	65.9	78.7	86.0		96.0		95.6									
		BUPI-HCl	06.3.30		70	81.1	75	106	10	56.1	67.0	77.4	84.1		92.0		96.6									
12-Apr-02		BUPI-HCl	11.3.30		70	77.5	75	106	10	81.0	90.4	93.9	94.1		93.6	96.2					90.2					
		BUPI-HCl	06.3.30		70	71.1	106	150	10	54.6	69.5	84.5	89.5		90.5		90.4									
12-Apr-02		BUPI-HCl	11.3.30		70	59.3	150	212	10	16.5	27.7	35.9	42.7		63.7	78.6					105.2					
18-May-02	44.59	BUPI-HCl	11.3.30		70	64.6	150	250	10	27.0	38.9	55.2	65.7		80.2	90.2					84.3					
		BUPI-HCl	06.3.30		70		150	212	10	19.5	27.5	40.7	51.7		79.1		80.9									
01-Jun-03	66.2	BUPI-HCl	11.3.30	25.6	70	57.2	150	250	10	20.5	34.6	43.4	49.7		78.1						118.0	22	AMB	NONE	1	02-Jun-02
01-Jun-03	66.2	BUPI-HCl	11.3.30	23.4	70	57.4	150	250	10	-19.9	-33.0	-40.8	-47.4		-78.3						93.8	22	AMB	AMB	1	02-Jun-02
01-Jun-03	66.2	BUPI-HCl	11.3.30	24.6	70	62.4	150	250	10	22.8	35.3	44.3	49.7		75.9						110.1	40	AMB	NONE	1	02-Jun-02
		BUPI-HCl	06.3.30		70		212	250	10	19.7	26.7	37.7	46.5		77.2		84.4									
12-Apr-02		BUPI-HCl	11.3.30		70	57.3	212	300	10	-9.0	-16.0	-26.1	-33.4		-56.1	65.1					96.5					
12-Apr-02		BUPI-HCl	11.3.30		70	57.6	300	425	10	6.8	12.6	21.3	29.6		48.7	86.4					105.4					
12-Apr-02		BUPI-HCl	11.3.20		80	76.0	75	106	10	92.9	98.4	100.0	100.5		100.7	100.7					100.7					
		BUPI-HCl	06.3.20		80		75	106	10	98.0	102.0	103.9	103.5		103.5		103.4									
		BUPI-HCl	06.3.20		80		106	150	10	49.0	67.1	82.3	86.9		96.0		95.8									
		BUPI-HCl	06.3.20		80		150	212	10	27.1	38.2	56.1	69.0		84.7		86.9									
12-Apr-02		BUPI-HCl	11.3.20		80	67.2	150	212	10	38.6	53.5	63.7	70.3		90.5	97.4					97.4					
09-Aug-03	66.92	BUPI-HCl	06.3.20	26.0	80	67.3	150	212	10	44.5	59.3	85.8	103.0		131.0						136.0	4	SEAL	NONE	2	15-Jul-01
09-Aug-03	66.92	BUPI-HCl	06.3.20	22.0	80	67.3	150	212	10	53.3	69.4	98.1	116.0		132.0						136.0	4	AMB	NONE	2	15-Jul-01
29-Jul-02	43.51	BUPI-HCl	06.3.20	23.0	80	82.0	150	212	10	39.6	51.2	73.9	88.5		91.8						94.9	22	AMB	AMB	1	15-Jul-01
29-Jul-02	43.51	BUPI-HCl	06.3.20	22.5	80	78.6	150	212	10	36.4	50.6	75.3	91.2		97.2						100.9	22	AMB	NONE	1	15-Jul-01
01-Aug-03	66.78	BUPI-HCl	06.3.20	21.9	80	72.3	150	212	10	53.0	73.0	95.6	111.0		121.0						126.0	22	AMB	NONE	2	15-Jul-01
01-Aug-03	66.78	BUPI-HCl	06.3.20	25.2	80	72.3	150	212	10	98.0	98.1	97.8	111.0		120.0						125.0	22	AMB	AMB	2	15-Jul-01
29-Jul-02	43.51	BUPI-HCl	06.3.20	19.5	80	78.9	150	212	10	36.4	54.0	76.1	88.2		92.0						95.1	40	AMB	NONE	1	15-Jul-01
09-Aug-03	66.92	BUPI-HCl	06.3.20	23.6	80	67.3	150	212	10	80.5	89.3	106.0	122.0		130.0						142.0	40	SEAL	NONE	2	15-Jul-01
01-Aug-03	66.78	BUPI-HCl	06.3.20	20.6	80	72.3	150	212	10	65.0	87.2	106.0	117.0		123.0						128.0	40	AMB	NONE	2	15-Jul-01
12-Apr-02		BUPI-HCl	11.3.20		80	62.7	212	300	10	19.1	34.4	52.0	61.4		84.6	93.2					101.4					
		ETID-HCl	11.2.30				38	75	10	47.5	71.0	91.5	103.0		103.0											
		ETID-HCl	06.2.50		50		75	106	10	6.0	9.1	12.3	15.1		26.2											
		ETID-HCl	06.2.50		50		106	150	10	2.4	3.9	8.1	9.8		26.5											
		ETID-HCl	06.2.50		50		150	212	10	3.9	6.1	10.6	13.3		24.3											
18-May-02		ETID-HCl	06.2.50		50	50.2	150	212	10	3.8	6.9	12.1	16.0		33.5	43.3					88.2	22	AMB	NONE	1	29-Apr-01
18-May-02		ETID-HCl	06.2.50		50	48.7	150	212	10	3.3	4.3	9.7	12.9		32.5	42.1					89.7	22	AMB	AMB	1	29-Apr-01
01-Jun-03	66.2	ETID-HCl	06.2.50	22.7	50	50.7	150	212	10	2.1	6.6	10.7	17.3		33.6						43.7	22	AMB	NONE	2	29-Apr-01
01-Jun-03	66.2	ETID-HCl	06.2.50	24.1	50	45.6	150	212	10	2.5	6.8	13.5	20.1		43.3						48.9	22	AMB	AMB	2	29-Apr-01
01-Jun-03	66.2	ETID-HCl	06.2.50	23.1	50	45.2	150	212	10	3.4	8.4	12.7	18.7		37.0						49.0	40	AMB	NONE	2	29-Apr-01
18-May-02		ETID-HCl	06.2.50		50	45.6	150	212	10	0.6	2.8	9.5	14.1		37.1	43.1					92.4	40	AMB	NONE	1	29-Apr-01
		ETID-HCl	06.2.50		50		212	300	10	2.9	4.7	8.5	12.4		30.8											
		ETID-HCl	06.2.40		60		75	106	10	20.9	28.8	40.7	51.2		82.4	82.7										
		ETID-HCl	06.2.40		60		150	212	10	5.3	8.1	12.5	14.6		18.0											

DATE	MG.Pg	DRUG	CODE	WEIGHT	NOM	ACTUAL	SIZE LOW	SIZE HIGH	SIZE IO	% DRUG RELEASE 1	2	4	6	12	24	48	72	96	120	144	TOTAL	STORAGE TEMP	HUMID	LIGHT	TIME YRS.	DATE STORED
		ETID-HCl	06.2.40		60		212	300	10	8.3	14.0	21.8	26.9		24.6											
		ETID-HCl	11.4.30		70	48.0	75	106	10	37.0	58.0	76.0	89.0		107.0						1115.0					
30-Mar-02		ETID-HCl	11.2.30		70	61.3	75	106	10	36.2	42.7	58.3	71.8		100.2	107.1					1112.6					
		ETID-HCl	06.2.30		70		75	106	10	17.1	23.5	38.7	48.5		70.1	80.0										
18-May-02	44.59	ETID-HCl	11.2.30		70	65.0	106	300	10	21.0	32.1	48.2	58.0		99.4	100.9					1105.9					
		ETID-HCl	11.4.30		70	49.0	106	150	10	16.0	22.0	34.0	45.0		84.0						1107.0					
01-Jun-03	66.2	ETID-HCl	11.2.30	25.0	70	64.7	106	300	10	13.0	25.2	38.0	48.6		82.0						1 99.0	22	AMB	AMB	1	02-Jun-02
01-Jun-03	66.2	ETID-HCl	11.2.30	24.8	70	64.4	106	300	10	25.4	39.4	55.6	68.0		97.9						1101.4	22	AMB	NONE	1	02-Jun-02
01-Jun-03	66.2	ETID-HCl	11.2.30	24.7	70	63.9	106	300	10	18.2	35.3	50.2	63.3		92.8						1117.3	40	AMB	NONE	1	02-Jun-02
		ETID-HCl	06.2.30		70		150	212	10	4.8	6.1	8.9	9.9		12.9											
		ETID-HCl	11.4.30		70	47.0	150	212	10	6.0	10.0	20.0	22.0		56.0						1104.0					
30-Mar-02		ETID-HCl	11.2.30		70	57.3	150	212	10	18.0	20.9	26.1	40.3		85.1	105.0					1113.8					
		ETID-HCl	06.2.30		70		212	300	10	17.7	19.4	29.3	35.1		41.2	42.3										
30-Mar-02		ETID-HCl	11.2.30		70	55.7	212	300	10	9.7	16.0	27.1	38.5		100.0	109.0					1115.1					
		ETID-HCl	11.4.30		70	43.0	212	300	10	4.0	5.0	13.0	15.0		41.0						1100.0					
30-Mar-02		ETID-HCl	11.2.30		70	58.7	300	425	10	21.2	22.6	42.7	53.5		95.9	100.5					1104.7					
		ETID-HCl	11.4.30		70	41.0	300	425	10	4.0	6.0	15.0	19.0		48.0						1105.0					
30-Mar-02		ETID-HCl	11.2.20		80	71.2	75	106	10	20.3	39.6	74.4	84.1		103.0	105.7					1108.5					
		ETID-HCl	11.4.20		80	58.0	75	106	10	32.0	53.0	69.0	84.0		101.0						1106.0					
		ETID-HCl	11.4.20		80	59.0	106	150	10	21.0	31.0	55.0	68.0		89.0						1 95.0					
		ETID-HCl	06.2.20		80		150	212	10	34.2																
		ETID-HCl	11.4.20		80	62.0	150	212	10	13.0	22.0	39.0	53.0		86.0						1 94.0					
30-Mar-02		ETID-HCl	11.2.20		80	67.9	150	212	10	20.9	32.0	60.9	85.5		105.0	107.9					1113.3					
30-Mar-02		ETID-HCl	11.2.20		80	67.4	212	300	10	25.3	27.7	46.0	62.6		90.0	109.0					1114.2					
		ETID-HCl	11.4.20		80	60.0	212	300	10	8.0	15.0	35.0	54.0		95.0						1105.0					
		ETID-HCl	11.4.20		80	62.0	300	425	10	9.0	14.0	27.0	42.0		93.0						1103.0					
27-Aug-02	54.00	ETID-HCl	11.4.10		90	65.0	75	106	10	52.0	81.0	96.0	104.0		105.0						1114.0					
27-Aug-02	54.00	ETID-HCl	11.4.10		90	65.0	106	150	10	36.0	58.0	73.0	84.0		106.0						1109.0					
27-Aug-02	54.00	ETID-HCl	11.4.10		90	68.0	150	212	10	29.0	48.0	62.0	78.0		95.0						1 97.0					
27-Aug-02	54.00	ETID-HCl	11.4.10		90	69.0	212	300	10	23.0	45.0	66.0	80.0		97.0						1 99.0					
27-Aug-02	54.00	ETID-HCl	11.4.10		90	70.0	300	425	10	27.0	50.0	78.0	92.0		104.0						1106.0					
27-Jul-03	66.60	LIDO-BASE	11.1.30	25.0	70	68.2	150	300	10	81.0	97.0	102.0	103.0		104.0						1109.0	40	SEAL	NONE	1	02-Jun-02
20-Jan-03	66.24	LIDO-BASE	11.1.30	20.4	70	65.9	212	300	10	27.2	40.5	61.8	73.4		97.6						1101.9	22	AMB	NONE	1	02-Jun-02
20-Jan-03	66.24	LIDO-BASE	11.1.30	27.7	70	65.3	212	300	10	20.7	42.8	61.9	73.1		98.4						1103.0	22	AMB	AMB	1	02-Jun-02
05-Nov-01		LIDO-BASE	11.1.30	24.4	70	90.5	75	106	10	86.3	94.5	99.7	100.1		101.9			100.4			1110.0					
05-Nov-01		LIDO-BASE	11.1.30	21.8	70	75.1	150	212	10	45.5	60.2	77.3	83.7		93.4			97.0			1105.0					
12-Nov-01		LIDO-BASE	11.1.30	71.7	70	71.6	212	300	10	16.0	23.7	36.6	44.7		79.2			88.8								
20-Jan-03	66.24	LIDO-BASE	11.1.30	23.5	70	71.0	212	300	10	91.6	95.6	95.2	94.8		95.4						1 98.0	40	AMB	NONE	1	02-Jun-02
05-Nov-01		LIDO-BASE	11.1.30	63.0	70	67.8	300	425	10	7.0	11.4	21.6	27.9		69.1			96.2			1111.0					
05-Nov-01		LIDO-BASE	11.1.20	27.1	80	93.7	75	106	10	92.6	99.2	101.4	102.2		103.8						1112.0					
05-Nov-01		LIDO-BASE	11.1.20	33.4	80	81.6	150	212	10	90.5	92.0	96.5	97.2		99.1			99.8			1107.0					
12-Nov-01		LIDO-BASE	11.1.20	90.7	80	80.9	212	300	10	76.2	95.1	97.4	98.4		100.3			100.7								
12-Nov-01		LIDO-BASE	11.1.20	89.8	80	82.4	300	425	10	46.8	69.2	88.5	90.5		92.8			93.5								
05-Nov-01		LIDO-BASE	11.1.10	27.7	90	93.5	75	106	10	99.0	97.1	101.5	102.1		103.9						1112.0					
05-Nov-01		LIDO-BASE	11.1.10	18.8	90	85.9	150	212	10	94.8	97.6	102.3	102.8		104.0						1111.0					
12-Nov-01		LIDO-BASE	11.1.10	59.9	90	88.7	300	425	10	66.6	87.5	95.6	97.3		97.5			98.6								
		LIDO-HCl	06.1.50		50	49.6	106	125	10	88.4	90.8	95.1	97.0													
		LIDO-HCl	06.1.50		50	46.0	125	150	10	62.0	76.3	85.7	88.5		90.3											
18-May-02	44.67	LIDO-HCl	06.1.50	12.7	50	50.0	150	212	10	41.0	53.5	71.1	79.7		85.1	85.4					1 92.6					
		LIDO-HCl	06.1.50		50	46.7	150	212	10	36.3	53.6	72.3	81.5		83.3											
09-Aug-03	66.92	LIDO-HCl	06.1.50	22.5	50	43.3	150	212	10	48.5	65.1	84.1	91.5		97.3						1104.0	4	SEAL	NONE	2	29-Apr-01
09-Aug-03	66.92	LIDO-HCl	06.1.50	23.4	50	43.3	150	212	10	44.2	61.3	83.3	90.0		97.1						1104.0	4	AMB	NONE	2	29-Apr-01

DATE	NO.Pg	DRUG	CODE	WEIGHT	MGM	ACTUAL	SIZE LOW	SIZE HIGH	% DRUG RELEASE 10	1	2	4	6	12	24	48	72	96	120	144	STORAGE TOTAL	TEMP	HUMID	LIGHT	TIME YRS.	DATE STORED
18-May-82	44.67	LIDO-HCl	06.1.50	15.5	50	49.1	150	212	10	41.4	55.4	71.4	77.0		82.7	83.4					89.8	22	AMB	NONE	/	29-Apr-81
20-Jun-83	66.68	LIDO-HCl	06.1.50	20.6	50	43.3	150	212	10	48.6	62.0	85.3	90.6		97.8						104.7	22	AMB	AMB	2	29-Apr-81
18-May-82	44.67	LIDO-HCl	06.1.50	12.7	50	43.7	150	212	10	47.4	61.8	82.1	89.7		98.3	98.6					105.2	22	AMB	AMB	/	29-Apr-81
20-Jun-83	66.68	LIDO-HCl	06.1.50	15.9	50	41.5	150	212	10	53.0	62.3	80.1	92.8		103.0						109.8	22	AMB	NONE	2	29-Apr-81
27-Jul-83	66.68	LIDO-HCl	06.1.50	22.1	50	43.3	150	212	10	64.6	77.4	88.8	93.2		96.4						102.6	40	SEAL	NONE	2	29-Apr-81
20-Jun-83	66.24	LIDO-HCl	06.1.50	16.9	50	45.1	150	212	10	70.8	78.5	93.9	92.4		96.1						101.2	40	AMB	NONE	2	29-Apr-81
18-May-82	44.67	LIDO-HCl	06.1.50	16.4	50	50.0	150	212	10	59.9	71.3	80.7	82.3		83.9	83.9					88.5	40	AMB	NONE	/	29-Apr-81
		LIDO-HCl	06.1.50		50	45.2	250	300	10	26.9	40.7	58.6	75.5		89.4	90.4										
		LIDO-HCl	06.1.50		50	44.8	425	600	10	33.8	50.0	69.3	81.2		91.3	92.2										

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